PROCEEDINGS

26TH INTERNATIONAL CONGRESS OF THE MEDITERRANEAN FEDERATION FOR HEALTH AND PRODUCTION OF RUMINANTS

FeMeSPRum

Novi Sad (Serbia), 20th – 23rd June, 2024

ZBORNIK RADOVA

26. MEĐUNARODNI KONGRES MEDITERANSKE FEDERACIJE ZA ZDRAVLJE I PRODUKCIJU PREŽIVARA

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Novi Sad (Srbija), 20. – 23.jun 2024.godine

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Dear Participants of the FeMeSPRum Congress,

Welcome to the 26th Congress of the Mediterranean Federation for Health and Production of Ruminants (FeMeSPRum). It is an honor to gather with you esteemed veterinarians and animal scientists dedicated to advancing the health and productivity of ruminants in the Mediterranean region.
This year's Congress is in the beautiful city of Novi Sad, Serbia. Nestled on the banks of the Danube River, Novi Sad is renowned for its vibrant culture, rich history, and stunning architecture. Known as the "Serbian Athens," it is home to the majestic Petrovaradin Fortress, numerous museums, galleries, and the lively Danube Park. As the European Capital of Culture for 2022, Novi Sad offers a perfect blend of tradition and modernity, providing a picturesque and inspiring backdrop for our meeting.
This year's Congress will focus on critical topics that directly impact the health, production, and welfare of the animals we care for. Topics include Biosecurity and heat stress on ruminant farms, Parasite control in ruminants, and Clinical pathology and healthcare of ruminants. These scientific sessions will provide cutting-edge insights and innovative solutions, besides fostering collaboration and the exchange of expertise among leading professionals from the region.
The Mediterranean region has a unique climate, geography, and agricultural practices that present specific challenges and opportunities for ruminant health and production. Advancing the health of domestic ruminants in this region is crucial for ensuring sustainable agriculture, enhancing food security, and supporting the livelihoods of countless farmers and communities. Your work and dedication play a vital role in addressing these challenges and promoting the well-being of domestic ruminant populations.
The Mediterranean Federation for Health and Production of Ruminants (FeMeSPRum) is an organization with immense potential. Its core idea is to serve as a medium for fruitful collaboration among stakeholders in ruminant production. This platform is not only for exchanging information and good practices but also aims to provide a consortium that can cooperate in writing international project proposals and succeed in international project calls. By working together, we can be more innovative and have an impact in our field. With this in mind, I am sure this Congress will boost this idea and strengthen our Federation.
All this would not be possible without the dedicated organizing committee and especially Prof. Dr. Marko Cincović, president of the organizing committee, who have done their best to prepare everything for a smooth congress. Your hard work and dedication are deeply appreciated. Additionally, thank you to all our sponsors, whose generous support has made this event possible.
Your participation and contributions to the Congress are not only crucial to the success of this Congress but also to the existence of the Federation. Together, we will explore new strategies, share best practices, and pave the way for significant advancements in ruminant health and production.
Thank you for being here, and I look forward to a productive and inspiring congress in the charming city of Novi Sad.
With best wishes,

Prof. Dr. Jože Starič
President of the Mediterranean Federation for Health and Production of Ruminants (FeMeSPRum)

Drage koleginice i kolege,
Mediteranska federacija za zdravlje i proizvodnju preživara (FeMeSPRum) je međunarodno udruženje koje okuplja različite profesionalce iz akademske i istraživačke sfere (najčešće veterinare, ali i agronome, inženjere animalne proizvodnje i dr.) koji su posvećeni brizi o preživarama, proučavanju i prevenciji bolesti ovih životinja, kao i povećanju i poboljšaju njihove proizvodnje (meso, mleko, vuna, itd.), dobrobiti i svega onoga što će uticati na dobijanje kvalitetnog i zdravstveno bezbednog proizvoda za krajnjeg potrošača. FeMeSPRum promoviše organizovanje obuka, diskusija, seminara i konvencija, sa definisanom periodičnošću, i podržava sva dešavanja koja doprinose unapređenju ovog sektora i saradnji između zemalja članica, a njeni direktni korisnici su stručna lica iz oblasti veterinarske medicine ali i drugih srodnih oblasti. Kao što mu ime govori, sfera uticaja se proteže na nekoliko zemalja mediteranskog regiona, uključujući Italiju, Španiju, Grčku, Tursku, Sloveniju, Hrvatsku, Siriju, Egipat, Tunis, Maroko. Iako naziv federacije ukazuje na njenu geografsku pripadnost, u eri globalne razmene i unapređenog transfera znanja i pomeranja klimatskih pojaseva, FeMeSPRum je proširio svoje delovanje i na zemlje u okruženju, a posebno značajna zemlja za ovu organizaciju je Srbija. U Srbiji smo 2011.godine imali kongres u Beogradu, a ove 2024.godine kongres se održava u Novom Sadu koji, na naše zadovoljstvo, organizujemo zajedno sa dve partnerske respektabilne ustanove i to su Departman za veterinarsku medicinu Novi Sad i Udruženje veterinara praktičara Srbije.
Dobro došli!

Prof. dr Jože Starič
Predsednik Mediteranske federacije za zdravlje i produkciju preživara (FeMeSPRum)

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FeMeSPRum Novi Sad (Serbia), 20th – 23rd June, 2024

ZBORNIK RADOVA 26. MEĐUNARODNI KONGRES MEDITERANSKE FEDERACIJE ZA ZDRAVLJE I PRODUKCIJU PREŽIVARA
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VETERINARY REVIEW / VETERINARSKI PREGLED

Predmet časopisa: Časopis objavljuje naučne i stručne radove iz oblasti veterinarske medicine i srodnih disciplina.
Jezik radova: Srpski (sa engleskim apstraktom) ili Engleski (sa srpskim sažetkom) po želji autora.
Periodičnost: Jedna sveska godišnje
Vrste radova: Originalni radovi, Pregledni radovi, Meta-analize, Radovi iz istorije veterinarske medicine, Radovi iz oblasti veterinarskog obrazovanja, Prikaz slučaja, Prikaz tehničkih rešenja, Prikazi knjiga, Naučna kritika, Pisma uredništvu, Izveštaji sa seminara i drugih stručnih događaja. Format: Časopis izlazi u štampanoj/elektronskoj formi i biće publikovan na sajtu Departmana za veterinarsku medicinu
Prijava radova na mejl: vetpregled@gmail.com

Subject of the Journal: The Journal publishes scientific and professional papers in the field of veterinary medicine and related disciplines.
Language: Serbian (with English abstract) or English (with Serbian abstract).
Periodicity: One No per year
Papers: original papers, review articles, meta – analysis, papers from the history of veterinary medicine, papers in the field of veterinary education, case report, technical solutions, book, scientific polemic, letters to the Editor, reports from seminars and other professional events.
For m at: The journal is published in printed and electronic form and will be published on the website of the Department of Veterinary Medicine.
Manuscript submission: vetpregled@gmail.com

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CONGRESS PROGRAMME / PROGRAM KONGRESA

20.06.2024. Welcome day / Dan dobrodošlice

21.06.2024. Workshop day / Dan radionica

22.06.2024. Main congress day / Glavni kongresni dan

23.06.2024. Conclusion day / Zaključci sa kongresa

20.06.2024. Welcome day / Dan dobrodošlice

17:00
Welcome cocktail, museum and city tour /
Koktel dobrodošlice i organizovani obilazak Muzeja Vojvodine i centra Novog Sada

21.06.2024. Workshop day / Dan radionica
Mesto održavanja ProMedia/ProReady, Kikinda

9:00
Bus departure in front of the Veterinary Clinic of the Department of Veterinary Medicine (corner of Cara Lazar Boulevard and Dr. Zoran Đinđić Street) / Polazak autobusa ispred Veterinarske klinike Departmana za veterinarsku medicinu (ugao Bulevara Cara Lazara i ulice dr Zorana Đinđića)

10:30-13:30
Radonica 1
Jovanović M., Cincović M.–
Proizvodnja gotovih mikrobioloških hranljivih podloga, nutrient supplement mediuma i virusnih transportnih medium sistema – od farme ovnova do fabričkog pogona, kontrole i sistema kvaliteta / Production of ready-made microbiological nutrient media, nutrient supplement medium and viral transport medium systems - from the ram farm to the factory plant, quality control and quality system

14:00-17:00
Radionica 2
Hristov S., Stanković B., Rađenović M. –
Biosigurnosne prakse na farmama preživara i kako komunicirati sa farmerima / Educational participatory workshop with stakeholders: biosecurity practices on ruminant farms and how to communicate it to farmers

17:00
Lunch for workshop participants sponsored by ProMedia Kikinda / Ručak za učesnike radionica sponzor ProMedia Kikinda

22.06.2024. Main congress day / Glavni kongresni dan
Mesto održavanja: Poljoprivredni fakultet Novi Sad –
Departman za veterinarsku medicinu, svečana sala P1,
Trg Dositeja Obradovića 8, 21000 Novi Sad

8:30-10:00
Registration / Registracija

10:00-10:15
Opening ceremony / Otvaranje kongresa

Area: Biosecurity and stress on ruminant farms / Oblast: Biosigurnost i stres na farmama preživara

10:15-10:30
Prodanov-Radulović J., Nunes T., Chantziaras I., Kureljušić B., Piccirillo A., Tamminen L-M., Niemi J., Rodrigues da Costa M., Allepuz A. -
COST action BETTER (CA20103) – an interdisciplinary research network in biosecurity in different animal production systems / COST akcija BETTER (CA20103) – interdisciplinarna mreža istraživača u oblasti biosigurnost u različitim proizvodnim sistemima životinja

10:30-10:45
Hristov S., Stanković B., Starič J., Nakov D., Prodanov Radulović J., Milovanović B., Chantziaras I., Allepuz A. -
Biosecurity measures on ruminant farms / Biosigurnosne mere na farmama preživara

10:45-11:00
Stanković B., Bugarski D., Ninković M., Kureljušić B., Kjosevski M., Chantziaras I. -
Implementation of biosecurity measures in ruminants farms / Primena biosigurnosnih mera na farmama preživara

11:00-11:15
Majkić M., Spasojević J., Nikolić S., Cincović M. -
Monitoring of heat stress in dairy cows – striving towards better resilience / Monitoring toplotnog stresa kod krava – stremljenje ka boljoj rezilijenciji

11:15-11:30
Čukić A., Cincović M., Djoković R., Rakonjac S., Petrović M., Petrović Ž.M.-
Heat stress impact on sheep production / Uticaj toplotnog stresa na proizvodnju ovaca

Area: Antiparasitic protection of ruminants / Oblast: Antiparazitska zaštita preživara

11:30-11:45
Bosco A., Nappa A., Capezzuto G., Lucibelli S., Nocerino M., Di Donato L., Vastolo A., Kiatti D., Calabrò S., Cutrignelli M.I., Sotiraki S., Rinaldi L. -
Anthelmintic efficacy of agro-industrial by-products against gastrointestinal nematodes of sheep: in vitro tests / Antihelmintička efikasnost nusproizvoda agroindustrije protiv gastrointestinalnih nematoda ovaca: in vitro testovi

11:45-12:00
Štrbac F., Stojanović D. -
Anthelmintic resistance in gastrointestinal nematodes of sheep: current situation and novel strategies / Antihelmintička rezistencija kod gastrointestinalnih nematoda ovaca: aktuelna situacija i nove strategije

12:00-13:00
Coffee break and poster section (see the list of works from the poster section on the next page) / Kafe pauza i poster sekcija (spisak radova iz poster sekcije pogledati na sledećoj strani)

Area: Clinical pathology and healthcare of ruminants / Oblast: Klinička patologija i zdravstvena zaštita preživara

13:00-13:15
Starič J., Veren Geč L., Marzel R., Ježek J. -
Sporadic leukosis in cattle / Sporadična leukoza kod goveda

13:15-13:30
Kurćubić V., Dmitrić M., Živković S., Petrović M.Ži. -
Severe adverse impact of bovine viral diarrhea on cattle production: a comprehensive approach to control / Štetni uticaj bovine virusne dijareje na proizvodne karakteristike goveda: sveobuhvatan pristup kontroli

13:30-13:45
Djoković R., Petrović Ž.M., Anđelić B., Čukić A. -
Lipidemia and lipid contents in the liver in holstein cows during transition period / Lipidemija i sadržaj lipida u jetri kod holstein krava u peripartalnom tranzivionom periodu

13:45-14:00
Ježek J., Starič J., Veren Geč L., Marzel R., Mrkun J. -
A relationship between serum copper concentrations and haematological and biochemical parameters in sheep / Veza između koncentracija bakra u serumu i hematoloških i biohemijskih parametara kod ovaca

14:00-14:15
Toholj B., Spasojević B., Kureljušić B., Galić I., Zahirović N. -
Ulcerative mamary dermatitis at dairy cows – etiology, prevalence, gross pathology and staging / Ulcerativni mamarni dermatitis mlečnih krava – etiologija, prevalenca i patologija

14:15-14:30
Nakov D., Zlatanovska B., Kocaleva Vitanova M., Miteva M., Hristov S., Stanković B. -
Mathematical modeling and machine learning prediction for prevalence dynamics of clinical mastitis in dairy herds / Matematičko modeliranje i predviđanje mašinskog učenja za dinamiku prevalencije kliničkog mastitisa u mlečnim stadama

14:30-14:45
Spasojević J., Cincović M., Majkić M., Stanojević J., Gurdeljević M., Gurjanov V. -
The use of ultrasound diagnostics and infrared thermography in diagnosis of limb diseases in cattle /Upotreba ultrazvučne dijagnostike i infracrvene termografije u dijagnostici oboljenja ekstremiteta kod goveda

15:00
Dodela nagrada i povelja i zatvaranje kongresnog dana / Awards ceremony and closing of the congress day
Informacije o e-sertifikatima/ Information about e-certificates
Ručak za sve učesnike/ Lunch for all participants

Poster sekcija

1. *Anđelić B., Djoković R., Cincović M., Majkić M. -*
   Influence of environmental factors and biological characteristics of cows on the fatty acid groups and nutritional index of raw milk / Uticaj faktora sredine i bioloških karakteristika krava na grupe masnih kiselina i indeks hranljivosti sirovog mleka

2. *Anđušić Lj., Milankov Z., Marić D.M., Milošević B., Djoković R., Maksimović G., Milanović V., Čukić A.-*
   Bioinformatička stimulacija lučenja oksitocina i njegova veza sa statusom kalcijuma, kalijuma, natrijuma i proizvodnjom mleka / Bioinformatics stimulation of oxytocin secretion and its relationship with calcium, potassium and sodium status and milk production

3. *Biricik H.S.- EU project on animal rescue from flood in Turkiye / Projekat EU o spasavanju životinja od poplava u Turskoj*

4. *Blond B., Majkić M., Spasojević J., Radinović M., Stanojević J. –*
   Infracrvena termografija – varijabilnosti i korelacije izmerenih temperatura kod krava / Infrared thermography - variability and correlations of measured temperatures in cows

5. *Cincović M., Starič J., Ježek J., Majkić M., Nikolić S., Blond B., Obradović N., Kovačević D. –*
   Krvna slika kod krava u peripartalnom periodu i odnos prema pokazateljima metaboličkog stresa / Complete blood count in cows in the peripartal period and the relationship to the indicators of metabolic stress

6. *Čukić A., Milošević B., Savić Ž., Anđušić Lj. –*
   Variability of morphological and growth characteristics of the Wurttemberg sheep breed / Varijabilnost morfoloških i osobina porasta Wirtemberg rase ovaca

7. *Delić Vujanović B., Došenović Marinković M., Đokić S., Djoković R., Petrović M., Majkić M.*
   Varijabilnost laboratorijskih parametara metaboličkog profila u ketozi kod krava – da li je dovoljno samo poređenje sa referentnim vrednostima? / Variability of laboratory parameters of the metabolic profile in ketosis in cows - is only comparison with reference values sufficient?

8. *Došenović Marinković M., Delić Vujanović B., Cincović M., Grubač S., Djoković R. –*
   Povezanost glukoze, insulina, nefa i indeksa insulinske rezistencije sa metaboličkom adaptacijom krava u periodu pre i posle teljenja / The relationship of glucose, insulin, nefa and index of insulin resistance with the metabolic adaptation of cows in the period before and after calving

9. *Grubač S., Cincović M., Starič J., Došenović Marinković M., Delić Vujanović B., Prodanov Radulović J. –*
Povezanost metabolizma gvožđa, organskih materija i flebotomije sa eritropezom preživara / The relationship of the metabolism of iron, organic matter and phlebotomy with the erythropoiesis of ruminants

10. *Hodnik J.J., Jankovec M., Ježek J., Krusic Ž., Mitterhofer S., Starič J. –*
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INFLUENCE OF ENVIRONMENTAL FACTORS AND BIOLOGICAL CHARACTERISTICS OF COWS ON THE FATTY ACID GROUPS AND NUTRITIONAL INDEX OF RAW MILK

UTICAJ FAKTORA SREDINE I BIOLOŠKIH KARAKTERISTIKA KRAVA NA GRUPE MASNIH KISELINA I INDEKS HRANLJIVOSTI SIROVOG MLEKA

Biljana Anđelić[1*], Radojica Djoković[2], Marko Cincović[3], Mira Majkić[3]

[1]University of Niš, Faculty of Agriculture, Kosančićeva 4, 37000 Kruševac, Serbia
[2]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, 32000 Čačak, Serbia
[3]University of Novi Sad, Faculty of Agriculture, Square Dositeja Obradovića 8, 21000 Novi Sad, Serbia
*Corresponding author: andjelic.biljana@ni.ac.rs

B.A. https://orcid.org/0000-0002-3976-0539, R.DJ. https://orcid.org/0000-0003-0900-3227, M.C. https://orcid.org/0000-0002-5683-8983, M.M. https://orcid.org/0009-0007-0110-7327

ABSTRACT

Cow´s milk contains a large number of fatty acids, some of which may be of potential benefit to human health. The aim of this research is to determine the most important biological and ecological factors that influence the fatty acid composition and nutritional indices of raw milk originating from cows in intensive farm production. Comparing to reference cows, next animals had significantly higher odds of having greater SFA concentrations and lower odds of being in the categories with higher MUFA, PUFA and UFA and concentrations in milk: cows in 2nd and 3rd lactation compared to 1st, cows in mid and late lactation compared to early, cows in heat stress compared to cows in thermoneutral period. Comparing to reference cows, cows with higher BHB had significantly lower odds of having greater SFA concentrations and higher odds of being in the categories with higher MUFA. Also, cows with higher NEFA had significantly lower odds of having greater SFA concentrations and higher odds of being in the categories with higher PUFA. In experimental group compared to reference we found lower odds of having greater nutrition index of milk. Odds to have higher nutrition index increase in cows with intense ketogenesis and lipolysis. In conclusion, older cows, cows in an advanced stage of lactation and cows in heat stress have higher values of SFA, while cows with higher lipolysis and ketogenesis have higher contents of MUFA, PUFA and UFA, which leads to a change in the nutritional indices of raw milk.

Key words: cows, fatty acids, nutritional index, parity, lactation period, heat stress, metabolic adaptation.

SAŽETAK

Kravlje mleko sadrži veliki broj masnih kiselina, od kojih neke mogu biti od potencijalne koristi za ljudsko zdravlje. Cilj ovog istraživanja je utvrđivanje najvažnijih bioloških i ekoloških faktora koji utiču na sastav masnih kiselina i nutritivne indekse sirovog mleka poreklom od krava u intenzivnoj farmskoj proizvodnji. U poređenju sa referentnim kravama, sledeće životinje su imale značajno veće šanse da imaju veće koncentracije SFA i manje šanse da budu u kategorijama sa većim MUFA, PUFA i UFA i koncentracijama u mleku: krave u 2. i 3. laktaciji u poređenju sa 1., krave u srednjoj i kasna laktacija u poređenju sa ranom, krave u toplotnom stresu u poređenju sa kravama u termoneutralnom periodu. U poređenju sa referentnim kravama, krave sa višim BHB su imale značajno manje šanse da imaju veće koncentracije SFA i veće šanse da budu u kategorijama sa većim MUFA. Takođe, krave sa višim NEFA imale su značajno niže šanse da imaju veće koncentracije SFA i veće šanse da budu u kategorijama sa većim PUFA. U eksperimentalnoj grupi u poređenju sa referentnom smo pronašli niže šanse za veći indeks nutritivnosti mleka. Šanse da se poveća nutritivni indeks je veći kod krava sa intenzivnom ketogenezom i lipolizom. Zaključno, starije krave, krave u poodmakloj laktaciji i krave u toplotnom stresu imaju veće vrednosti SFA, dok krave sa većom lipolizom i ketogenezom imaju veći sadržaj MUFA, PUFA i UFA, što dovodi do promene indeksa hranljivosti sirovog mleka.

Ključne reči: krave, masne kiseline, nutritivni indeks, paritet, period laktacije, toplotni stres, metabolička adaptacija.

INTRODUCTION

Burr was the first who described fatty acids, in the early 1930s. He was also the first who identified linoleic acid (C18: 2 n6) and alpha linolenic acid (C18: 3 n3), important fatty acids in pigs and rats, as essential fatty acids for growth, skin structural health and reproduction in their research (1). Later, the significance of fatty acids have been shown by many researchers. They are lipid mediator molecules, such as prostaglandins, prostacyclins, thromboxanes, leukotrienes, liposines, and resolvins, which have influence on cellular function. Santos et al. (2) stated that fatty acids which body takes are included in the phospholipids in cell membranes to determine the structural and functional properties of the cells. Fatty acids (FA) are used in animals for many reasons: to grow, to have a healthy lifestyle, to maintain a fertile lactation period and to increase fertility. Since cells in mammals can not synthesize some fatty acid groups, mammals must take those from the outside with feed. Fatty acids are hydrophobic or amphipathic molecules and they have the functions of forming structural components of cell membranes, synthesizing prostaglandins and attaching proteins to cell membranes. What is more, fatty acids are also stored as intracellular triacylglycerides (TAG) in lipid droplets and they provide a powerful source of energy when the body needs it. Fox and Mc Sweeney (3) stated that cow milk is made up mostly of water (around 87.7%), proteins (3.3%), fat (3.4%), carbohydrates (4.9%), and vitamins and minerals (0.7%). Since it is directly connected with the flavor and chemical-physical properties of milk and dairy products, the economic value of fat is the greatest among all milk constituents (4). She also noted that different milk fatty acids compose nearly 90% of the milk fat weight. As reported, some fatty acids here are related to human health problems (4).

The FA catalogue includes saturated fatty acids (SFAs), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA), regarding the number of double bonds. As explained by Barlowska and Litwińczuk (5), milk fat contains a complex mixture of various lipid substances and these lipids are primarily triglycerides (triacylglycerides). By weight, they make up 98% of the total milk fat. Other milk lipids are diacylglycerides (0.25- 0.48%), monoacylglycerides (0.02-0.04%), phospholipids (0.6-1.0%), cholesterol (0.2-0.4%), glycolipids (0.006%) and free fatty acids (0.1-0.4%). With their unique physico-chemical and biological properties, the triclycerides in milk comprise over 400 different fatty acids (5). Siegel and Ermilov (6) further explained that biologically active lipid substances are primarily monounsaturated fatty acids (MUFAs), oleic acid (C18:1 n-9), polyunsaturated fatty acids (PUFAs), linoleic acid (LA; C18:2 n-6) and α-linolenic acid (ALA; C18:3 n-3). Precursors of eicosanoids are PUFAs with 20C, mainly docosahexaenoic acid (DHA; C20:5 n-3) and eicosapentaenoic acid (EPA; C22:6 n-3) and they regulate various physiological processes (6).

Furthermore, milk is an important source of saturated FA, especially whole milk and high-fat dairy products like cream or butter. Many authors have claimed that saturated FA is unhealthy because of the relation between saturated fat intake and cardiovascular disease. Yet, this has been recently brought into question by new research (7). There is an evidence that dietary exposure to whole dairy products can considerably affect several health conditions, even chronic diseases, by reducing risk in later life (8,9). Still, it is not clear if saturated FA are harmful or not to human health, and therefore the use of low-cost indexes that may better characterize the diet in human population studies is timely. For instance, Briggs et al. (10) stated that some human diseases are associated with high concentrations of saturated fatty acids, while on the other hand, many beneficial characteristics of high levels of unsaturated fatty acids have also been proven (11).

There is a large number of fatty acids (FA) in bovine milk, such as polyunsaturated fatty acids (PUFA) in the n-3 (omega-3) FA group and the conjugated linoleic acid (CLA) isomer *cis*-9 *trans*-11 C18:2. These may be potentially beneficial for humans (12). There have been great efforts to change the composition of milk in order to provide a better source of PUFA and reduce the saturated FA

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(SFA) component (13), and for this reason it is necessary to determine seasonal, herd-level management, as well as the nutritional factors that influence milk FA composition. This will allow the formulation of recommendations for producers who are aiming to enhance the content of beneficial FA in milk.The FA profile of bovine milk depends both on the consumed FA and on biohydrogenation processes of FA in the rumen. Therefore, the FA composition of bovine milk is affected by many factors, such as breed, season, geographical location, as well as access to fresh grazing, grazing sward type, silage type, cereal feeding, and oil supplementation of feed (14).

In order to estimate the effect of diet on cardiovascular health (CVH), PUFA/SFA is an index that is normally used. It is theorized that all PUFAs in the diet can depress low-density lipoprotein cholesterol (LDL-C) and lower levels of serum cholesterol, while all SFAs contribute to high levels of serum cholesterol. For this reason, when this ratio is higher, the effect is more positive (15). It seems that the PUFA/SFA ratio for itself is not enough to predict the change of plasma cholesterol level, since a large amount of dietary MUFA may lead to an increase of lipids in blood plasma and liver (on rats model). It appears that the prerequisites for keeping low plasma and liver cholesterol are low MUFA/SFA ratio, high PUFA/MUFA ratio and PUFA + MUFA/SFA ratio not to exceed 2 (16), and this relation is confirmed in modern experimental survey (17).

The aim of this research is to determine the most important biological and ecological factors that influence the fatty acid composition and nutritional indices of raw milk originating from cows in intensive farm production.

MATERIAL AND METHODS

The study was carried out from July 2019 to June 2022 in Vojvodina region in order to collect data for a complete three year. The data used in the study were obtained from 350 cows (all of which are the Holstein breed) belonging to 15 dairy farms selected by simple random sampling. FA composition included the quantity of SFA, MUFA, PUFA and UFA (in g/100 g of milk fat) was performed using Fourier-transform infrared (FTIR) spectrometry (Milkoscan FT6000, Foss Analytics, Hilleroed, Denmark). Information about cows included: parity (1, 2, 3 or higher), lactation period (early, mid, late), ambient factor (thermoneutral period, heat stress period), cows blood ketone (optimal ketogenesis and high ketogenesis with BHB ≥1,1 mmol/L) and NEFA (optimal lipolysis and high lipolysis with NEFA ≥0,7 mmol/L). BHB and NEFA were determined by standard colorimetric assay and on automatic spectrophotometer (Chemray, Rayto, China).

The concentrations of each FA and group and ratio were divided into four categories according to the quartiles of its distribution. Descriptive values were determined as position values for SFA, MUFA, PUFA, UFA, UFA/SFA, MUFA/SFA and PUFA/MUFA as follows: lower quartile, lower median quartile, median value, upper median quartile and upper quartile. In addition to the descriptive values, the correlation between the investigated fatty acids and nutritional indices was determined. In a multivariate approach, mixed-effect ordinal regression models were used to estimate the influence of explanatory variables (parity, lactation period, ambient temperature, blood BHB and NEFA) on the concentration of FA group (SFA, MUFA and PUFA) as dependent variables. Analysis performed across FA and nutritional index quartiles. The regression coefficients indicate the likelihood of being in a higher category when the explanatory variables change. Descriptive values, correlation analysis and model analysis were performed using SPSS statistical package (IBM, USA).

RESULTS AND DISCUSSION

Saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and total unsaturated fatty acids (UFA) (g/100 g fatty acids) quartile distributions in milk samples from dairy cows are presented in Table 1. Median value (min-max) for selected parameters was: 70,7 (65,1-74,5) for SFA; 25,2 (21,5-28,7) for MUFA; 3,31 (2,65-3,82) for PUFA; 28,51 (24,1-32,5) for UFA; 0,40 (0,37-0,43) for UFA/SFA; 0,356 (0,32-0,372) for MUFA/SFA; 0,046 (0,04-0,051) for PUFA/SFA and 0,131 (0,12-0,133) for PUFA/MUFA.

Cow milk fat typically contains 60-70% SFA and in the majority of mammals' milk fat the main SFA is palmitic acid (C16:0) (18,19). Milk fat, declared as one of the most complex natural fats, is made up of nearly 400-500 fatty acids (5, 20). Milk PUFAs are only ~3% of all fatty acids (21), while percentage of monounsaturated fatty acids (MUFA) is almost the same in sheep, cow, and goat milk fat

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and may vary from about 20% to approximtely 35% (22-24). Considering a negative role of the C12:0, C14:0, and C16:0 acids, authors proposed atherogenic indices (AI) and thrombogenic indices (TI) (25). Based on AI and TI values conclusions may be brought concerning fat quality from the point of view of human diet. The values of AI and TI of ruminant milk could be improved by the administration of either olive cake, rapeseed oil, linseed oil, or camelina sativa cake to the diet (26). The results about nutritive index are in accordance with previous results of many authors (27-30).

Correlation analyzes show a high and significant negative correlation between SFA and other FA groups, as well as nutritional indices. MUFA, PUFA and UFA show a positive correlation with each other. The nutritional indices of UFA/SFA, MUFA/SFA, PUFA/SFA showed significant correlations with each other, but not with the PUFA/MUFA index. The results are shown in Table 2. Correlation analysis of Fa in milk, blood, urine and feces in cows showed significant correlation between selected FA in different biological materials (31).

Table 1. Fatty acids in raw milk (g/100g offat) and nutritive indexes

	SFA	MUFA	PUFA	UFA	UFA/SFA	MUFA/SFA	PUFA/SFA	PUFA/MUFA
Lower quartile	65.1-67.0	21.5-23.5	2.65-2.80	24.1-26.3	0.37-0.38	0.320-0.341	0.040-0.042	0.120-0.127
Lower median quartile	67.1-70.5	23.6-25.1	2.81-3.30	26.4-28.4	0.381-0.399	0.342-0.355	0.043-0.045	0.123-0.129
Median	70.7	25.2	3.31	28.51	0.40	0.356	0.046	0.131
Upper median quartile	70.11-72.0	25.3-26.9	3.32-3.50	28.52-30.5	0.41-0.42	0.357-0.368	0.047-0.049	0.131-0.132
Upper quartile	72.1-74.5	27.0-28.6	3.51-3.82	30.51-32.50	0.421-0.43	0.369-0.372	0.050-0.051	0.131-0.133

Table 2. Correlation of fatty acid groups and nutritive indexes

	SFA	MUFA	PUFA	UFA	UFA/SFA	MUFA/SFA	PUFA/SFA	PUFA/MUFA
SFA	1							
MUFA	-0.79**	1						
PUFA	-0.84**	0.76**	1					
UFA	-0.96**	0.82**	0.91**	1				
UFA/SFA	-0.995**	0.71**	0.84**	0.99**	1			
MUFA/SFA	-0.996**	0.785**	0.86**	0.87**	0.81**	1		
PUFA/SFA	-0.998**	0.75**	0.999**	0.89**	0.85**	0.8**	1	
PUFA/MUFA	0.26	-0.92**	0.93**	0.19	0.26	-0.28	-0.17	1

Comparing to reference cows, next animals had significantly higher odds of having greater SFA concentrations and lower odds of being in the categories with higher MUFA, PUFA and UFA and concentrations in milk: cows in 2nd and 3rd lactation compared to 1st, cows in mid and late lactation compared to early, cows in heat stress compared to cows in thermoneutral period. Comparing to reference cows, cows with higher BHB had significantly lower odds of having greater SFA concentrations and higher odds of being in the categories with higher MUFA. Also, cows with higher NEFA had significantly lower odds of having greater SFA concentrations and higher odds of being in the categories with higher PUFA. In experimental group compared to referent we found lower odds of having greater nutrition index of milk. Odds to have higher nutrition index increase in cows with intense ketogenesis (higher BHB) and lipolysis (higher NEFA). There was not found statistical significant change of odds for PUFA/MUFA index in function of examined factors.

The diet is responsible for 95% of the differences in cow milk fat (12, 14, 32, 33) and for that reason a group of authors claimed the diet is the crucial factor. Lock and Garnsworthy (34) showed seasonal changeability in the CLA content, which is measured via Δ9-desaturase activity. Similarly

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Elgersma et al. (35) noticed seasonal changes in cow milk CLA content between winter and summer.

In some studies 1st parity cows had higher proportions of UFA and oleic acids and lower propotions of SFA and C16:0 in milk fat, when compared to later parity cows. However, a number of these studies showed that 3rd parity cows had higher levels of milk PUFA and MUFA than 2nd parity cows (4, 36,37).

Auldist et al. (38) noted that fat yield is highest in summer and accordingly, lowest in winter. What is more, there were lower levels of SFA and higher amounts of MUFA and PUFA found in summer milk, especially C18:1, in comparison to winter (39, 40).

On the whole, higher proportions of MUFA were found in milk from cows in early lactation than in milk from medium or late lactation, especially C18:1 (54). The phase of lactation has a great influence - C14:0, C16:0, SFA and SCFA have a tendency to increase as lactation progresses, which our study also proved. These are usually higher in mid lactation, when compared to concentration in milk from early lactation. On the other hand, C18:0, C18:1, MUFA and PUFA tend to decrease as lactation progresses, with the lowest levels usually in mid lactation.

Several different studies find larger contents of MUFA and LCFA with ketosis due to the mobilization of FA during NEB, which our study also showed (41-43). Number of authors suggested that MUFAs, mainly represented by C18:1 *c*9, decrease in proportion until week 12 pp (44, 45). Significantly higher proportion of SFAs, which is noticed around day 150 pp, was associated with the later stages of lactation when animals were no longer in NEB (46, 47). Our experimental model and obtained results are in relation with the latest results of Rodríguez-Bermúdez et al (48) who compared the quartiles and found that season, health, lactation period and organolepctic characteristics of milk had significant effect on fatty acids.

There are two indexes that characterize the atherogenic and thrombogenic potential of the diet based on the content in saturated (SFA) and unsaturated FA, introduced by Ulbricht and Southage (31), in addition to the PUFA/SFA ratio. Indeed, some authors have pointed that the PUFA/SFA ratio might not be sufficient to evaluate the nutritional value of dietary fat, since it ignores the effects of MUFA. What is more, some SFA have no effect on plasma cholesterol (49). PUFA/SFA decreased during lactation, which is in relation with previous result obtained from Nantapo and coworkers (28) or presented in meta-review of Chen and Liu (15).

CONCLUSIONS

Concentration of fatty acids and nutritive index in raw milk of cows is in relation with previous finding from cows in intensive milk production, although we found lower median value of unsaturated and higher value of saturated fatty acids. The probability that cows have a higher or lower value of fatty acids depends on many factors such as the number of lactations (parity), lactation period, ambient temperature and metabolic adaptation of the cows. In general, each of the mentioned factors leads to an increase in the value of saturated fatty acids and a decrease in unsaturated fatty acids, except in the case of cows with increased lipid catabolism, i.e. poor metabolic adaptation, when the opposite changes are expected in milk. A good herd structure of the cow on a farm with balanced rations and good environmental conditions can contribute to the production of raw milk with a certain fatty acid composition.

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Table 3. Influence of biological and environmental factors on fatty acids and nutritive index of raw milk in cow

	SFA	MUFA	PUFA	UFA	UFA/SFA	MUFA/SFA	PUFA/SFA	PUFA/ MUFA
Age(Lactation 1 as control)								
Lactation 2	2.13** (1.89-2.23)	0.59** (0.47-0.73)	0.94 (0.88-1.12)	0.78** (0.65-0.86)	0.59** (0.41-0.71)	0.62** (0.45-0.79)	0.78 (0.56-1.41)	0.89 (0.8-1.19)
Lactation 3	1.86** (1.53-1.92)	0.73** (0.62-0.85)	0.89 (0.87-1.06)	0.82* (0.72-0.93)	0.71* (0.58-0.95)	0.53** (0.72-0.93)	0.89 (0.70-1.56)	0.85 (0.78-1.13)
Lactation period(Early lactation as control)								
Mid lactation	4.59** (4.11-4.86)	0.39** (0.25-0.43)	0.22** (0.18-0.26)	0.27** (0.26-0.31)	0.58** (0.51-0.66)	0.71** (0.69-0.85)	0.49** (0.42-0.56)	0.95 (0.81-1.1)
End lactation	4.21** (4.06-4.39)	0.41** (0.30-0.51)	0.26** (0.2-0.31)	0.32** (1.24-0.37)	0.62** (0.55-0.68)	0.73** (0.64-0.81)	0.58** (0.45-0.67)	0.91 (0.79-1.12)
Ambient temperature (Termoneutral period as control)								
Heat stress period	1.93** (1.78-2.06)	0.65** (0.55-0.71)	0.52** (0.46-0.58)	0.54** (0.48-0.57)	0.39** (0.36-0.47)	0.48** (0.41-0.55)	0.52** (0.45-0.58)	0.92 (0.84-1.09)
Ketogenesis (Normal ketogenesis as control)								
High ketogenesis	0.56** (0.45-0.61)	1.39** (1.16-1.48)	1.05 (0.93-1.14)	1.26** (1.21-1.32)	1.16** (1.09-1.21)	1.21** (1.14-1.32)	1.03 (0.89-1.14)	0.93 (0.75-1.08)
Lipolysis (Normal lipolysis as control)								
High lipolysis	0.41** (0.37-0.45)	1.12 (0.85-0.13)	2.33** (2.09-2.5)	1.56** (1.42-1.63)	1.69** (1.53-1.82)	1.29** (1.22-1.41)	2.06** (1.98-2.16)	0.89 (0.75-1.19)

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REFERENCES

1. *Burr G.O., Burr M.M. On the nature and role of the fatty acids essential in nutrition. Journal of Biological Chemistry 1930, 86, 587-621. https://doi.org/10.1016/S0021-9258(20)78929-5*
2. *Santos J.E.P., Greco L.F., Garcia M., Thatcher W.W., Staples C.R. The role of specific fatty acids on dairy cattle performance and fertility. Florida Ruminant Nutrition Symposium 2013, pp. 74-88.*
3. *Fox P.F., McSweeney P.L.H. Dairy chemistry and biochemistry. Blackie, London, 1998.*
4. *Samková E., Špička J., Pešek M., Pelikánová T., Hanuš O. Animal factors affecting fatty acid composition of cow milk fat: A review. South African Journal of Animal Science 2012, 42, 2, 83-100.*
5. *Barłowska J., Litwińczuk Z. Nutritional and pro-health properties of milk fat. Medycyna Weterynaryjna 2009, 65, 171-174.*
6. *Siegel G., Ermilov E. Omega-3 fatty acids: Benefits for cardio-cerebro-vascular diseases. Atherosclerosis 2012, 225. 2. 291–295. DOI:10.1016/j.atherosclerosis.2012.09.006*
7. *Siri-Tarino P.W., Sun Q., Hu F.B., Krauss R.M. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. The American Journal of Clinical Nutrition 2010, 91, 3, 535-546. https://dx.doi.org/10.3945%2Fajcn.2009.27725*
8. *Markey O., Vasilopoulou D., Givens D.I., Lovegrove J.A. Dairy and cardiovascular health: Friend or foe? Nutrition Bulletin 2014, 39, 161-171. DOI:10.1111/nbu.12086*
9. *Givens D.I. Milk Symposium review: The importance of milk and dairy foods. In the diets of infants, adolescents, pregnant women, adults, and the elderly. Journal of Dairy Science 2020, 103, 11, 9681-9699. https://doi.org/10.3168/jds.2020-18296.*

10. *Briggs M.A., Petersen S.K., Kris-Etherton P.M. Saturated Fatty Acids and Cardiovascular Disease: Replacements for Saturated Fat to Reduce Cardiovascular Risk. Healthcare 2017, 5, 2, 29. https://doi.org/10.3390/healthcare5020029*
11. *Haug A., Høstmark A.T., Harstad O.M. Bovine milk in human nutrition–a review. Lipids in health and disease 2007, 6, 1, 1-16. doi: 10.1186/1476-511X-6-25*
12. *Jensen R.G. The composition of bovine milk lipids: January 1995 to December 2000. Journal of Dairy Science 2002, 85, 295–350. doi: 10.3168/jds.S0022-0302(02)74079-4*
13. *Voigt J., Hagemeister H. Dietary influence on a desirable fatty acid composition in milk from dairy cattle. Journal of Animal and Feed Sciences 2001, 10, 87–103. DOI: https://doi.org/10.22358/jafs/70014/2001*
14. *Ellis K.A., Innocent G., Grove-White D., Cripps P., McLean W.G., Howard C.V., Mihm M. Comparing the fatty acid composition of organic and conventional milk. Journal of Dairy Science 2006, 89, 6, 1938-1950. DOI: 10.3168/jds.S0022-0302(06)72261-5*
15. *Chen J., Liu H. Nutritional Indices for Assessing Fatty Acids: A Mini-Review. International Journal of Molecular Sciences 2020, 8, 21, 16, 5695. doi: 10.3390/ijms21165695.*
16. *Chang N.W., Huang P.C. Effects of the ratio of polyunsaturated and monounsaturated fatty acid to saturated fatty acid on rat plasma and liver lipid concentrations. Lipids 1998, 33, 5, 481-487. doi: 10.1007/s11745-998-0231-9.*
17. *Zhu T., Lu X.T., Liu Z.Y., Zhu H.L. Dietary linoleic acid and the ratio of unsaturated to saturated fatty acids are inversely associated with significant liver fibrosis risk: A nationwide survey. Frontiers in Nutrition 2022, 26, 9, 938645. doi: 10.3389/fnut.2022.938645.*
18. *Markiewicz-Kęszycka M., Czyżak-Runowska G., Lipińska P., Wójtowski J. Fatty Acid Profile Of Milk - A Review. Bulletin of the Veterinary Institute in Pulawy 2013, 57, 135–139. DOI: 10.2478/bvip-2013-0026*
19. *Soyeurt H., Dardenne P., Gillon A., Croquet C., Vanderick S., Mayeres P., Bertozzi C., Gengler N. Variation in fatty acid contents of milk and milk fat within and across breed. Journal of Dairy Science 2006, 89, 4858–4865. https://doi.org/10.3168/jds.S0022-0302(06)72534-6*
20. *Månsson H.L. Fatty acids in bovine milk fat. Food & Nutrition Research 2008, 52. doi: 10.3402/fnr.v52i0.1821.*
21. *Devle H., Rukke E.O., Naess-Andresen C.F., Ekeberg D. A GC-magnetic sector MS method for identification and quantification of fatty acids in ewe milk by different acquisition modes. Journal of Separation Science 2009, 32, 3738-3745. DOI: 10.1002/jssc.200900455*
22. *Butler G., Stergiadis S., Seal C., Eyre M., Leifert C. Fat composition of organic and conventional retail milk in northeast England. Journal of Dairy Science 2011, 94, 24-36. doi: 10.3168/jds.2010-3331*
23. *O'Donnell-Megaro A., Barbano D., Bauman D. Survey of the fatty acid composition of retail milk in the United States including regional and seasonal variations. Journal of Dairy Science 2011, 94, 59-65. doi: 10.3168/jds.2010-3571*
24. *Schmidely P., Andrade P.V.D. Dairy performance and milk fatty acid composition of dairy goats fed high or low concentrate diet in combination with soybeans or canola seed supplementation. Small Ruminant Research 2011, 99, 135-142. https://doi.org/10.1016/j.smallrumres.2011.04.010*
25. *Ulbricht T., Southgate D. Coronary heart disease: seven dietary factors. Lancet 1991, 338, 985-992.*
26. *Szumacher-Strabel M., Cieślak A., Zmora P., PersKamczyc E., Bielińska S., Stanisz M., Wojtowski J. Camelina sativa cake improved unsaturated fatty acids in ewe's milk. Journal of the Science of Food and Agriculture 2011, 91, 2031-2037. doi: 10.1002/jsfa.4415.*
27. *Lobos-Ortega I., Pizarro-Aránguiz N., Urrutia N.L., Silva-Lemus M., Pavez-Andrades P., Subiabre-Riveros I., Torres-Püschel D. Determination of nutritional health indexes of fresh bovine milk using near infrared spectroscopy. Grasas y Aceites 2022, 73, 2, e458-e458. DOI: https://doi.org/10.3989/gya.0450211*
28. *Nantapo C., Muchenje V., Hugo A. Atherogenicity index and health-related fatty acids in different stages of lactation from Friesian, Jersey and Friesian×Jersey cross cow milk under a pasture-based dairy system. Food Chemistry 2014, 146, 127–133. doi: 10.1016/j.foodchem.2013.09.009.*
29. *Sinanoglou V.J., Koutsouli P., Fotakis C., Sotiropoulou G., Cavouras D., Bizelis I. Assessment of lactation stage and breed effect on sheep milk fatty acid profile and lipid quality indices. Dairy Science and Technology 2015, 95, 509–531. doi: 10.1007/s13594-015-0234-5.*
30. *Bonanno A., Di Grigoli A., Mazza F., De Pasquale C., Giosuè C., Vitale F., Alabiso M. Effects of ewes grazing sulla or ryegrass pasture for different daily durations on forage intake, milk production and fatty acid composition of cheese. Animal 2016, 10, 2074–2082. doi: 10.1017/S1751731116001130.*
31. *Anitaş Ö., Göncü S. Relations between feces, urine, milk and blood fatty acid contents in cattle. MOJ Ecology & Environmental Sciences 2018, 3, 6, 356-362.*
32. *Kelsey J.A., Corl B.A., Collier R.J., Bauman D.E. The effect of breed, parity and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. Journal of Dairy Science 2003, 86, 2588–2897. https://doi.org/10.3168/jds.S0022-0302(03)73854-5*

33. *Pietrzak-Fiećko R., Tomczyński R., Świstowska A., Borejszo Z., Kokoszko E., Smoczyńska K. Effect of mare's breed on the fatty acid composition of milk fat. Czech Journal of Animal Science 2009, 54 403–407. DOI: 10.17221/1683-CJAS*
34. *Lock A.L., Garnsworthy P.C. Seasonal variation in milk conjugated linoleic acid and ∆9-desaturase activity in dairy cows. Livestock Production Science 2003, 79, 47-59.DOI:10.1016/S0301-6226(02)00118-5*
35. *Elgersma A., Tamminga S., Ellen G. Modifying milk composition through forage. Animal Feed Science and Technology 2006, 131 207–225. https://doi.org/10.1016/j.anifeedsci.2006.06.012*
36. *Bilal G., Cue R.I., Mustafa A.F., Hayes J.F. Effects of Parity, Age at Calving and Stage of Lactation on Fatty Acid Composition of Milk in Canadian Holsteins. Canadian Journal of Animal Science 2014, 94, 401–410. doi: 10.4141/cjas2013-172.*
37. *Bastin C., Soyeurt H., Gengler N. Genetic Parameters of Milk Production Traits and Fatty Acid Contents in Milk for Holstein Cows in Parity 1–3. Journal of Animal Breeding and Genetics 2012, 130, 118–127. doi: 10.1111/jbg.12010.*
38. *Auldist M.J., Walsh B.J., Thomson N.A. Seasonal and Lactational Influences on Bovine Milk Composition in New Zealand. Journal of Dairy Research 1998, 65, 401–411. doi: 10.1017/S0022029998002970.*
39. *Collomb M., Bisig W., Bütikofer U., Sieber R., Bregy M., Etter L. Seasonal Variation in the Fatty Acid Composition of Milk Supplied to Dairies in the Mountain Regions of Switzerland. Dairy Science and Technology 2008, 88, 631–647. doi: 10.1051/dst:2008029.*
40. *Frelich J., Šlachta M., Hanuš O., Špička J., Samková E., Weglarz A., Zapletal P. Seasonal Variation in Fatty Acid Composition of Cow Milk in Relation to the Feeding System. Animal Science Paper and Reports 2012, 30, 219–229.*
41. *Mann S., Nydam D., Lock A.L., Overton T., McArt J. Short Communication: Association of Milk Fatty Acids with Early Lactation Hyperketonemia and Elevated Concentration of Nonesterified Fatty Acids. Journal of Dairy Science 2016, 99, 5851–5857. doi: 10.3168/jds.2016-10920.*
42. *Chandler T., Pralle R., Dórea J., Poock S., Oerzel G., Fourdraine R., White H. Predicting Hyperketonemia by Logistic and Linear Regression Using Test-Day Milk and Performance Variables in Early-Lactation Holstein and Jersey Cows. Journal of Dairy Science 2018, 101, 2476–2491. doi: 10.3168/jds.2017-13209.*
43. *Gross J., van Dorland H.A., Bruckmaier R.M., Schwarz F.J. Milk fatty acid profile related to energy balance in dairy cows. Journal of Dairy Research 2011, 78, 479–488. DOI: 10.1017/S0022029911000550*
44. *Mele M., Dal Zotto R., Cassandro M., Conte G., Serra A., Buccioni A., Bittante G., Secchiari P. Genetic parameters for conjugated linoleic acid, selected milk fatty acids, and milk fatty acid unsaturation of Italian Holstein-Friesian cows. Journal of Dairy Science 2009, 92, 392–400. DOI: 10.3168/jds.2008-1445*
45. *Stoop W.M., Bovenhuis H., Heck J.M.L., van Arendonk J.A.M. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. Journal of Dairy Science 2009, 92, 1469–1478. https://doi.org/10.3168/jds.2008-1468*
46. *Van Haelst Y.N.T., Beeckman A., Van Knegsel A.T.M., Fievez V. Short communication: Elevated concentrations of oleic acid and long-chain fatty acids in milk fat of multiparous subclinical ketotic cows. Journal of Dairy Science 2008, 91, 4683–4686. https://doi.org/10.3168/jds.2008-1375*
47. *Vranković L., Aladrović J., Octenjak D., Bijelić D., Cvetnić L., Stojević Z. Milk fatty acid composition as an indicator of energy status in Holstein dairy cows. Archives Animal Breeding 2017, 60, 205–212. https://doi.org/10.5194/aab-60-205-2017*
48. *Rodríguez-Bermúdez R., Fouz R., Rico M., Camino F., Souza T.K., Miranda M., Diéguez F.J. Factors Affecting Fatty Acid Composition of Holstein Cow's Milk. Animals (Basel) 2023. 13(4):574. doi: 10.3390/ani13040574.*
49. *Orellana C., Peña F., García A., Perea J., Martos J., Domenech V., Acero R. Carcass characteristics, fatty acid composition, and meat quality of CriolloArgentino and Braford steers raised on forage in a semi-tropical region of Argentina. Meat Science 2009. 81, 57-64. https://doi.org/10.1016/j.meatsci.2008.06.015.*

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BIOINFORMATIČKA STIMULACIJA LUČENJA OKSITOCINA I NJEGOVA VEZA SA STATUSOM KALCIJUMA, KALIJUMA, NATRIJUMA I PROIZVODNJOM MLEKA

BIOINFORMATICS STIMULATION OF OXYTOCIN SECRETION AND ITS RELATIONSHIP WITH CALCIUM, POTASSIUM AND SODIUM STATUS AND MILK PRODUCTION

Ljiljana Andjušić[1*], Zoran Milankov[2], Dušan M. Marić[3], Božidar Milošević[1], Radojica Djoković[4], Goran Maksimović[1], Valentina Milanović[1], Aleksandar Čukić[1], Željko Savić[1]

[1]Department of Animal Science, Faculty of Agriculture, Lešak, University of Priština, Serbia,
[2] Dental Clinic, 'Tusk'; Novi Sad, Serbia,
[3] Institute for child and mother health of Vojvodina, Faculty of Medicine, Novi Sad, University of Novi Sad, Serbia,
[4] Department of Animal Science, Faculty of Agronomy Čačak, University of Kragujevac, Serbia,
*Corresponding author: Dr Ljiljana Anđušić ljiljana.andjusic@pr.ac.rs

Lj.A. https://orcid.org/0000-0002-0054-1128, Z.M. https://orcid.org/0000-0001-8926-3445, D.M.M. https://orcid.org/0000-0002-3981-3215, B.M. https://orcid.org/0000-0003-4381-0276, R.Dj. https://orcid.org/0000-0003-0900-3227, G.M. https://orcid.org/0000-0001-8159-2818, V.M. https://orcid.org/0000-0002-7177-9643, A.Č. https://orcid.org/0000-0001-9063-3431, Ž.S. https://orcid.org/0009-0006-5932-3551

SAŽETAK

Bioinformatika je nauka koja se bavi bioinformacionim procesima i pojavama u živim ćelijama pri informacionom delovanju svetlosti – elektromagnetnih talasa, jonizujućeg zračenja, bakterija i virusa, biološki aktivnih i materija hemijske prirode. Elektromagnetno polje (EMF) predstavlja protok energije u formi električnog i magnetnog polja koji zajedno čine EM talas. Ove dve komponente EM talasa osciluju u fazi normalno jedna na drugu i upravno na pravac prostiranja energije. U biološkim sistemima električni fenomeni su predstavljeni električnim i jonskim strujama, i njih izazivaju i prenose joni. U bioelektrične fenomene spadaju transmembranski potencijali i to mirovni membranski potencijal, akcioni potencijal i lokalni potencijali. Na ekscitabilnost ćelije utiču koncentracije K^+ i Ca^{2+} u estracelularnoj tečnosti. Oslobađanje oksitocina (OT) je Ca^{2+} zavisni akt koji je normalno izazvan depolarizacijom sekretornih završetaka pod uticajem akcionog potencijala od magnocelularnih neurosekretornih neurona. Oslobađanje OT delimično nastaje zbog smanjene K^+ provodljivosti, i povećanog priliva Ca^{2+} jona, ističu isti autori. Elektrofiziološki snimci pokazuju da, neposredno pre svakog istiskivanja mleka, čitava neurosekretorna OT populacija stvara sinhronizovanu eksploziju akcionih potencijala što dovodi do oslobađanja OT iz nervnih završetaka neurohipifize Mlečnost krava može biti kontrolisana putem bioinformacionih signala odnosno povećanje mlečnosti može biti izazvano-stimulisano elektromagnetnim talasom – informacijom. Nakon bioinformatičke stimulacije mleko ne menja svoje biološke osobine. Pokazano je da varijacije u koncentraciji hormona i elektrolita pod dejstvom EMF mogu dovesti do fizioloških promena i uticati pozitivno na proizvodnju mleka. Razumevanje bioinformatičkih procesa i tehnologija može biti od velike važnosti u procesu povećanja proizvodnje mleka kod krava, posebno ovakve stimulacije ne bi trebale da imaju bilo kakve neželjene efekte.

Ključne reči: krave, biostruje, elektromagnetno polje, joni, oksitocin, proizvodnja mleka.

ABSTRACT

Bioinformatics is a science that deals with bio-informational processes and phenomena in living cells during the informational action of light - electromagnetic waves, ionizing radiation, bacteria and viruses, biologically active and chemical substances. Electromagnetic field (EMF) is a flow of energy in the form of an electric and magnetic field that together make up an EM wave. These two components of the EM wave oscillate in phase normal to each other and perpendicular to the direction of energy propagation. In biological systems, electrical phenomena are represented by electrical and ionic currents, and they are caused and carried by ions. Bioelectrical phenomena include transmembrane potentials, i.e. resting membrane potential, action potential and local potentials. Cell excitability is influenced by the concentrations of $K+$ and $Ca^{2}+$ in the extracellular fluid. Oxytocin (OT) release is a $Ca^{2}+$ dependent act that is normally triggered by action potential depolarization of secretory terminals from magnocellular neurosecretory neurons. The release of OT is partly due to reduced $K+$ conductivity and increased influx of $Ca^{2}+$ ions, the same authors point out. Electrophysiological recordings show that, immediately before each milk ejection, the entire neurosecretory OT population creates a synchronized explosion of action potentials, which leads to the release of OT from the nerve endings of the neurohypophysis. The milk production of cows can be controlled by means of bio-informational signals, that is, the increase in milk production can be induced-stimulated by an electromagnetic wave - information. After bioinformatic stimulation, milk does not change its biological properties. It has been shown that variations in the concentration of hormones and electrolytes under the influence of EMF can lead to physiological changes and have a positive effect on milk production. Understanding bioinformatics processes and technologies can be of great importance in the process of increasing milk production in cows, especially if stimulation of the cows should not have any side effects.

Key words: cows, biocurrents, electromagnetic field, ions, oxytocin, milk production

BIOINFORMATIKA I ELEKTROMAGNETNO POLJE

Bioinformatika (grč. *Bios* – život; engl. *Informatics*) je nauka koja se bavi bioinformacionim procesima i pojavama u živim ćelijama pri informacionom delovanju svetlosti – elektromagnetnih talasa, jonizujućeg zračenja, bakterija i virusa, biološki aktivnih i materija hemijske prirode. Prvi bioinformatički programi su bili razvijeni za DNK sekvencijalnu analizu. Poslednja naučna bioinformatička istraživanja usmerena su na ćeliju i međućelijske komunikacije. Više decenija unazad veoma aktivno se proučavaju principi delovanja bioloških materija na izolovane organe kod ljudi i životinja, sa posebnim naglaskom na informaciono stanje ćelija. Utvrđeno je da hemijski molekuli utiču na ćelijsku strukturu, kako neposrednim kontaktom, tako i indirektnim delovanjem. U svim eksperimentima, biološki efekat na ćelije je zavisio ne samo od doza postojećih materija i njihovih energija, već od kvaliteta informacije tj. informacione komponente. Ćelija je jasno reagovala na strukturu informacionog polja materije, pri čemu prenošenje signala nije zavisilo od količine (materijalne doze) hemijske supstance i energije.

Elektromagnetno polje (EMF) predstavlja protok energije u formi električnog i magnetnog polja koji zajedno čine EM talas. Ove dve komponente EM talasa osciluju u fazi normalno jedna na drugu i upravno na pravac prostiranja energije. U svetlu moderne kvantne teorije, elektromagnetno zračenje se definiše kao protok fotona koji se kroz vakuumski prostor kreću brzinom svetlosti ($c=3\times10^{8}$ m/s). Svaki od fotona nosi određenu energiju koja se povećava srazmerno povećanju frekvencije (1). Sposobnost recepcije magnetnog polja (MF) Zemlje zauzima posebno mesto među mnoštvom mehanizama pomoću kojih živi organizmi nastoje da dobiju informaciju od sveta koji ih okružuje. Poznato je da geomagnetno polje (GMF) može uticati na migraciju životinja i njihovo ponovno vraćanje (2). Živa bića se rađaju i rastu u prisustvu više fizičkih polja, kao što su gravitacijsko i geomagnetno polje (3). Pored toga, istraživanja (4-6) pokazuju da, drugi celularni elementi poput mikrotubula, igraju važnu ulogu u interakciji između ćelije i EM polja. Mikrotubule su komponente citoskeleta i sastoje se od tubulina. To su cilindrični polimeri 25 nm u prečniku i 100 nm dužine. Mikrotubule imaju učešća u kretanju, rastu, obliku ćelija, kao i u organizaciji funkcija unutar ćelija (uključujući i neurone) (**7**). Tubulin proteini koje sadrže mikrotubule sastavljeni su od alfa i beta monomera, koji stvaraju dipole što dovodi do feroelektričnih svojstava (8). Zbog toga, mikrotubule su visoko polarne (električne) strukture koje omogućavaju proizvodnju EM polja (8,9). Dalje, izlaganje MF može indukovati efekte u mikrotubularnoj organizaciji (10). Veoma kratko izlaganje MF izazvalo je samoorganizovanje u mitohondrijama, koje su centrala mnogih funkcija u ćeliji. Sposobnost životinja u korišćenju informacija

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GMF u orjentaciji i navigaciji je dobro dokumentovana, i poznata kao magnetorecepcija (11). Više laboratorijskih ogleda svedoče da, društveni insekti, kao što su pčele i mravi, mogu koristiti magnetno polje kao informaciju u svojoj orjentaciji (12).

BIOSTRUJE, JONI I NEUROENDOKRINI ODGOVOR OKSITOCINA

Poznato je da biostruje nastaju kao posledica električne aktivnosti ćelija, posebno mišićnih i nervnih. Električno aktivan deo ćelije, koji transformiše hemijsku energiju u električnu, je ćelijska membrana. Procesi, koji protiču u njoj, definišu sliku biomagnetskih polja, ili kako se kaže, biomagnetskih signala. Bioelektrični fenomeni su kontrolni sistemi koji sinhronizuju funkcije najvećeg broja ćelija. U biološkim sistemima električni fenomeni su predstavljeni električnim i jonskim strujama, i njih izazivaju i prenose joni. U bioelektrične fenomene spadaju transmembranski potencijali i to mirovni membranski potencijal, akcioni potencijal i lokalni potencijali. Na ekscitabilnost ćelije utiču koncentracije K^+ i Ca^{2+} u estracelularnoj tečnosti.

Treba istaći da joni kalcijuma (Ca^{2+}) veoma utiču na aktivaciju i deaktivaciju Na- i K- kanala, što se pokazalo delovanjem na mišićne i nervne ćelije. Smatra se da je i u osnovi ovog procesa promena konformacije lipoproteinskog kompleksa kanala pod uticajem jona Ca^{2+} (13). Aktivacija ćelijske membrane predstavlja otvaranje potencijal zavisnih, transmiter zavisnih i drugih jonskih kanala (14). Stimulacija ćelijske membrane može biti električne, toplotne, mehaničke, hemijske i druge prirode. Kada akson stimulišemo električnim stimulusom otvaraju se Na^+ kanali u Ranvijerovom čvoru, što omogućava difuziju Na^+ jona u ćeliju, dok joni K^+ izlaze iz ćelije tj. i jedni i drugi joni (Na^+ i K^+) kreću se iz sredine veće koncentracije u sredinu niže koncentracije (koncentracioni gradijent). Unutrašnja površina membrane na mestu nadražaja postaje pozitivna, a spoljašnja negativna, što dovodi do promene vrednosti membranskog potencijala (od -70mV prema 0). Taj proces se naziva *depolarizacija membrane*. Kod aksona se vrednost menja od -85mV ka +30mV. Izlučivanje mleka iz mlečne žlezde je urođeni refleks koji se javlja kao odgovor na taktilnu stimulaciju mlečnih žlezda kroz neuroendokrini refleksni luk (15). Istiskivanje mleka je aktivni transport alveolarnog mleka u odeljak cisterne (16). Stimulacijom receptora na papilama, impulsi se prenose aferentnim vlaknima preko kičmene moždine do mozga , tačnije do paraventrikularnog i supraoptičkog jezgra hipotalamusa, u kojima je sintetizovan oksitocin. OT se potom transportuje do zadnjeg režnja hipofize (neurohipofize), odakle se oslobađa u cirkulaciju (17). Putem krvi oksitocin dospeva do oksitocin-receptora na mioepitelnim ćelijama koje okružuju mlečne alveole i smeštene su duž mlečnih kanalića. Ova interakcija hormon-receptor izaziva kontrakciju mioepitelnih ćelija, koje potiskuju mleko iz alveola i kanalića u cisternu mlečne žlezde (18), odnosno, povećava se pritisak po okviru mlečne žlezde i nastaje ejekcija mleka (19). Unutar hipotalamusnih jezgara, isti neuroni koji proizvode OT primaju ekscitatorne signale holinergičkih neurona za prenos signala do hipofize. Ova sekvenca predstavlja eferentnu komponentu neuroenodokrinog refleksa. Oslobađanje OT je Ca^{2+} zavisni akt koji je normalno izazvan depolarizacijom sekretornih završetaka pod uticajem akcionog potencijala od magnocelularnih neurosekretornih neurona. Oslobađanje OT delimično nastaje zbog smanjene K^+ provodljivosti, i povećanog priliva Ca^{2+} jona, ističu isti autori. Elektrofiziološki snimci pokazuju da, neposredno pre svakog istiskivanja mleka, čitava neurosekretorna OT populacija stvara sinhronizovanu eksploziju akcionih potencijala što dovodi do oslobađanja OT iz nervnih završetaka neurohipifize (15,16).

KONTROLA MLEČNOSTI POMOĆU BIOINFORMACIONIH SIGNALA I UTICAJA NA KONCENTRACIJU OKSITOCINA, Na, K I Ca

Mlečnost krava može biti kontrolisana putem bioinformacionih signala odnosno povećanje mlečnosti može biti *izazvano-stimulisano elektromagnetnim talasom – informacijom.* Nakon bioinformatičke stimulacije mleko ne menja svoje biološke osobine. Razumno je pretpostaviti da, eventualne varijacije u koncentraciji ispitivanih parametara (hormona i elektrolita) mogu dovesti do fizioloških promena.

DNK je biološki internet. Biološki prenosioci informacija nisu samo materijalne prirode, već su to i energetski prenosioci. Ti energetski signalni mehanizmi, kao npr. elektromagnetne frekvence, mogu biti stotinu puta efikasniji u prenošenju podataka iz okoline u odnosu na hemijske signale, kao što su hormoni, neurotransmiteri, faktori rasta i sl. OT, hormon zadnjeg režnja hipofize, poseduje nekoliko fizioloških tj. biohemijskih aktivacionih efekata, tokom kojih izaziva kontrakciju mioepitala mišićnih ćelija oko mlečnih kanalića, izazivajući ispuštanje mleka. Aferentno, neurobiofeedback-om se posle nekog vremena opet izaziva ispuštanje OT i tako ukrug, sve dok su zadovoljeni prirodni fiziološki principi u tom smislu. Aktivacija hipofizalnog OT zavisi od aferentnih nervnih impulsa, koji se generišu akcionim potencijalom generisanim u ćelijama. Akcioni potencijal je unutarćelijski doprinos fizioloških radnji ćelije. Te fiziološke radnje se dešavaju pri aktivaciji ćelijskih receptora. Ćelijski receptori se stvaraju tokom diferencijacije ćelije. Diferencijacija ćelija zavisi od ekspresije gena, tj. aktivnosti DNK. Aktivnost nervnih ćelija je s toga, filogenetski determinisana. Aktivacija receptora se dešava uz pomoć neurotransmitera. Svaka aktivacija putem neurotransmitera ostavlja elektromagnetski efekat. Aktivacija navedenog ćelijskog DNK se vrši preko biofeedback (bfb) sistema. Pri biofeedback aktivnostima emituju se fotoni koji imaju određena svojstva koja se mogu snimiti određenim načinom i sačuvati u bazi podataka (20). Pošto su ćelijski receptori u biofeedback sprezi, oni reaguju na specifičnu elektromagnetnu ekscitaciju, te automatski aktiviraju biofeedback, koji aferentno ekscitira hipofizu. Eletromagnetski efekat se detektuje u samoj ćeliji, kada se prepozna aktivacioni signal koji tako pokreće efekat akcionog potencijala, a koji se poznatim tokom prenosi aferentno do neurohipofize. Takav aferentni signal dovodi do aktivacije ćelija koje luče oksitocin. Upotrebom EM stimulatora aktiviraju se specifični receptori specifičnih tkiva, koji EM stimulacijom posredno aktiviraju DNK ćelije, a dalji tok stimulacije hipofize i lučenja oksitocina ide svojim prirodnim tokom. U prvoj fazi se na specifičan način vrši snimanje nervnih impulsa i čuvanje istih u bazi podataka. Oblik sačuvanih snimaka može biti digitalni i analogni. Taj postupak je predmet posebnog patenta. Svi nervni signali su u prirodi digitalni. Uređaj koji se koristi u prirodi je digitalni elektromagnetni stimulator specifičnih ćelijskih receptora. Koristi se Delta Sigma (DS) modulacioni princip aktivacije. DS modulator snimljeni analogni signal prevodi u digitalni oblik. Takav digitalni signal u takvom modulatoru dovodi do elektromagnetskog oscilovanja na frekvenciji od oko 400 kHz. Tako ta frekvencija postaje noseći talas snimljene digitalne biofeedback informacije.

DS modulator šalje signal koji se dovodi na *triger* kola 555, što dovodi do pobude izlaznog stimulišućeg kola 555. Kolo je spojeno sa antenskim kalemom 0,39 mH. Takav kalem, delujući kao antena, šalje elektromagnetni signal u prostor, kružno oko 10 m. Kalem, ili više njih, se postavlja kod ciljane životinje.

Ukoliko se u okolini nalazi više životinja, koristi se efekat interferentnog elektromagnetskog polja, gde se fenomen enterferencije koristi da se pokrije sve veći prostor elektromagnetskim poljem navedenih karakteristika.

Već duži niz godina se ispituje mehanizam interakcije između ekstremno niske frekvencije EMF i ćelijske membrane i bioloških sistema uopšte (21-24).

Tajmerom se reguliše doza EM zračenja, koja podrazumeva periode zračenja i periode pauze. Na ulaz DS modulatora se dovodi ekcitacioni signal koji u sebi sadrži modulisanu digitalnu informaciju, ranije snimljenu u prvoj fazi, prethodno i u knjizi navedenim postupkom (20). Na životinje se primenjuje jedinstven protokol bioinformatičke stimulacije u trajanju od 30 dana, sledećom dinamikom : 15 minuta stimulacija, 8 sati pauza. Uporedo sa EM stimulacijom čuje se muzika Baha i Mocarta. Takav ciklus stimulacije traje do kraja ogleda. Prvobitno, tj. prva šest dana su stimulatori bili postavljeni (15 uređaja) naizmenično po jedan dan (30 ekperimentalnih životinja) u predelu glave životinja.

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Slika 1. Postavljanje elektromagnetnog stimulatora i sakupljača talasa na životinje i u objektu

Nakon ovog perioda, uređaji su pričvršćeni za poprečnu metalnu šipku, postavljeni iznad glave životinja, na visini od oko 1m odstojanja. S obzirom da se u oglednoj grupi nalazi 30 životinja, koje se slobodno kreću u okviru objekta, uređaji su postavljeni tako da dovode do interferentnog pojačavanja emitovanog signala. Iskorišćeni su uslovi faznog pojačanja, tako da svi ćelijski receptori dobijaju dovoljan prag elektromagnetnog talasa, ali na informaciju reaguju samo oni receptori koji prepoznaju i demodulišu ulazni, ranije snimljeni, kodirani elektromagnetski emitovani interferentni talas.

Trend povećanja koncentracije oksitocina se uočava u toku trajanja oglednog perioda i nastavlja se sve do kraja i nakon perioda elektromagnetne stimulacije i to na nivou statističke značajnosti od 1%. 17-og dana ogleda, merenja su izvršena kod 30 grla u oglednoj i 30 grla u kontrolnoj grupi životinja, pa imamo drugačije tablične vrednosti oksitocina. Dobijena razlika od 52,9 pg/ml u korist ogledne grupe je statistički vrlo značajna, sa zabeleženom maksimalnom vrednosšću oksitocina 483,9 pg/ml u oglednoj grupi. Razlika u odnosu na period pre tretmana iznosi 49,1 pg/ml. Srednja vrednost oksitocina u oglednoj grupi životinja je iznosila 251,72 pg/ml, što je više za 61,71 pg/ml u odnosu na nivo oksitocina pre početka ogleda (190,01 pg/ml). Statistički veoma značajna razlika (164,9**) se javlja i 26 – dana oglednog perioda sa zabeleženom maksimalnom vrednošću oksitocina u oglednoj grupi od 595,0 pg/ml. Srednja vrednost oksitocina u oglednoj grupi iznosi 361,92, pri čemu je izračunata razlika u sadržaju oksitocina u odnosu na period pre stimulacije 171,1 pg/ml. Dva dana nakon perioda stimulacije nastavlja se trend povećanja koncentracije oksitocina u oglednoj grupi životinja, pri čemu je zabeležena maksimalna vrednost oksitocina od 596,7 pg/ml u oglednoj grupi i statistički vrlo značajna razlika između grupa na nivou od 1% (143,4**). Razlika u sadržaju oksitocina u odnosu na period pre ogleda iznosi 192,16 pg/ml. Nadalje, sve razlike koje se javljaju, u sadržaju oksitocina između ogledne i kontrolne grupe, su vrlo značajne, na nivou od 1% i to kod sva tri modela obračuna testa koji su korišćeni. Nakon završetka stimulacije (12 dana) trend povećanja sadržaja oksitocina nastavlja svoj rast, sa zabeleženom maksimalnom vrednošću u oglednoj grupi od 589,2 pg/ml i maksimalnom srednjom vrednošću od početka ogleda 383,98, što je za 193,97 pg/ml više u odnosu na period pre stimulacije. Dakle, elektromagnetnom stimulacijom, ne samo da je izvršena aktivacija hipofizalnog oksitocina, koji izaziva kontrakciju mioepitelnih mišićnih ćelija oko mlečnih kanalića, izazivajući ispuštanje mleka, već je i stvoren uslovni refleks, čime se i objašnjava povećanje koncentracije ovog hormona i nakon završetka oglednog perioda. Zabeležene su znatno veće vrednosti kod sva tri modela obračuna testa statističke značajnosti. Tendencija porasta nivoa oksitocina utvrđena u ovom ogledu, takođe je zabeležena u radu Lefcourt i saradnika (25), pri povremenoj stimulaciji, sa zebeleženim maksimalnim vrednostima kod većine tretiranih životinja od 350 pg/ml.

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Tabela 1. Koncentracija oksitocina (OT, pg/mL) na početku i na kraju ogleda

Nedelja	Grupa	Grla	Mean±SE	SD	CV%	min.-maks.	Razlik a
Pre tretmana	*ogled.*	60	190,01 12,6	65,7	34,7	67,6-323,8	3,8^{NS}
	kontr.	60	193,81 12,2	67,3	34,8	66,3-337,1	
UKUPNO	*ogled.*	180	293,30 17,8	99,4	33,9	61,2-596,7	84,2**
	kontr.	180	209,11 14,51	75,9	36,3	66,3-477,0	

Na^+ i K^+ imaju tendenciju porasta tokom čitavog perioda stimulacije kod gotovo svih oglednih životinja, i to na nivou statistički vrlo značajnih razlika ($p<0,01$) u odnosu na period pre tretmana. Razlika u sadržaju Na^+ pre i u toku ogleda od 18,4 mmol/l je statisčki vrlo značajna. Zabeležene maksimalne vrednosti Na^+ pre ogleda iznose 135 mmol/l i nalaze se na donjoj fiziološkoj granici**,** dok su vrednosti Na^+ u toku perioda stimulacije bile veće (140 mmol/l) i u granicama referntnih vrednosti i dosežu vrednosti gornje fiziološke granice (26-28). Minimalne vrednosti Na^+ su daleko ispod nivoa referentnih vrednosti. Prosečna koncentracija Na^+ u toku stimulacije (131,3 mmol/l) je bila veća u odnosu na vrednosti pre ogleda (112,9 mmol/l). Mala je razlika u sadržaju K^+ pre i u toku ogleda od 0,57 mmo/l, ali statistički vrlo značajna. Prosečna vrednost K^+ pre ogleda (3,52 mmol/l) se nalazi ispod donje fiziološke granice (26-28) dok je u periodu stimulacije nešto veća (4,09 mmol/l) i nalazi se na donjoj fiziološkoj granici. Zabeležene maksimalne vrednosti K^+ pre ogleda (4,8 mmol/l) se nalaze u granicama referentnih vrednosti, dok se u toku stimulacije (5,8 mmol/l) nalaze na gornjoj fiziološkoj granici (26-28).

Kod svih životinja u ogledu zabeleženo je statistički vrlo značajno povećanje nivoa Ca^{2+} u krvnom serumu krava u toku perioda stimulacije, što se nesumnjivo može pripisati uticaju EM stimulacije. To se poklapa sa rezultatima dobijenim u radu (29), kod Holštajn nebremenitih junica, gde su zabeležene gotovo isto veće vrednosti nivoa Ca^{2+}, ne samo u krvnoj plazmi, već i cerebrospinalnoj tečnosti u toku, i posle perioda tretmana. Evidentno je razlog tome uticaj EMF, ističu isti autori. Tendencija povećanja nivoa Ca^{2+} se javlja i u CSF, pri čemu krvno moždana barijera može biti izmenjena tokom perioda ekspozicije, omogućavajući veći priliv kalcijuma u CSF, kao što je prikazano u ogledima sa pacovima (30). Nakon izlaganja hipofize pacova magnetnom polju 50 Hz u trajanju od 30 min. zabeležen je povećan nivo Ca^{2+} jona (31). I u radu (32), takođe je uočeno povećanje nivoa Ca^{2+} u serumu izloženih pacova.

Na osnovu dobijenih vrednosti Ca^{2+} prikazanih u tabeli male su razlike u sadržaju Ca^{2+} pre i u toku ogleda od 0,34 mmol/l, ali statistički vrlo značajne. Prosečne vrednosti Ca^{2+} pre ogleda (1,77 mmol/l) se nalaze ispod donje fiziološke granice, dok se prosečne vrednosti Ca^{2+} u toku stimulacije (2,11 mmol/l) nalaze na donjoj granici fizioloških vrednosti (26-28). Maksimalne vrednosti Ca^{2+} pre ogleda (2,65 mmol/l) dosežu gornju fiziološku granicu, a u toku stimulacije (2,92 mmol/l) nivo Ca^{2+} premašuje gornje referentne vrednosti (26-28).

Na osnovu iznetih rezultata gore pomenutih ispitivanja, može se zaključiti da joni Ca^{2+}, Na^+ i K^+ igraju ključnu ulogu u odgovoru životinja na EM stimulaciju ELF, jer se promene dešavaju i na nivou jonskih kanala, što ima za posledicu aktivaciju Na^+/K^+ -ATPaze koja teži da uspostavi jonsku homeostazu i samim tim dodatnu potrošnju ATP-a (33). Imajući u vidu jonsku osnovu akcionog potencijala, koji se odvija preko aktivnosti voltažno zavisnih jonskih kanala za Na^+ i K^+, možemo pretpostaviti da je efekat primenjene EM stimulacije ostvaren na nivou ćelije.

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Tabela 2. Koncentracija Na^+, K^+ i Ca^{2+} pre i tokom ogleda

	Faza ogleda	Grla	X±Sx	SD	CV%	min.-maks.	Razlika
Na (mmol/l)	Pre	60	112,9±2,23	16,1	14,2	73-135	18,4**
	U toku	60	131,3±1,69	12,2	9,3	76-140	
K (mmol/l)	Pre	60	3,52±0,07	0,55	15,8	2,3-4,8	0,57**
	U toku	60	4,09±0,08	0,56	13,8	2,5-5,8	
Ca (mmol/l)	Pre	60	1,77±0,04	0,28	16,1	1,29-2,65	0,34**
	U toku	60	2,11±0,04	0,29	13,9	1,32-2,92	

Ustanovljena je i linearna korelativna povezanost između koncentracije oksitocina i ostalih elektrolita u krvi ispitivanih životinja, a rezultati sa regresionim jednačinama prikazani u tabeli 3. Može se iz podataka videti da su svi koeficijenti korelativne povezanosti ili negativni do pozitivno jako slabi, osim za povezanost između oksitocina i magnezijuma, gde praktično nema korelacije (-0,60) i bez statističke značajnosti. Koeficijenti korelativne povezanosti između ostalih elemenata su statistički značajni. Ako posmatramo koeficijent korelativne povezanosti između oksitocina (sa jedne strane), kalijuma i kalcijuma, sa druge, možemo uočiti da je statistički vrlo značajan.

Tabela 3. Koeficijenti korelativne povezanosti i regresione jednačine između koncentracije oksitocina i elektrolita u krvi ispitivanih krava Holštajn frizijske rase

Elektro-lit	Koeficijent korelacije *R*	Greška korelacije	*t*-vrednost	*p*-vrednost	Regresiona jednačina
Na^+	0,150*	0,012	1,534	0,03	*Na=0,313* x *Oxitocin*
K^+	0,243**	0,09	2,535	0,01	*K=0,01* x *Oxitocin*
Ca^{2+}	0,200**	0,01	2,060	0,01	*Ca=0,005* x *Oxitocin*

Zbirnim obračunom za oglednu (1.260) i kontrolnu (1.260) grupu životinja, možemo ustanoviti da je povećanje prinosa mleka u oglednoj grupi, u odnosu na kontrolnu grupu životinja, statički vrlo značajno (2,09**). Na osnovu podataka Glavne odgajivačke organizacije za AP Vojvodinu (2015) proizvodnja mleka na farmama AP Vojvodine, gde je 80% zastupljena holštajn frizijska rasa goveda, proizvodnja mleka je bila niža (20,32 kg) u odnosu na prosečne rezultate (31,10 kg) dobijene u ovom ogledu. Izloženost EM polja (60 Hz) imalo je uticaja na povećanje prinosa mleka od 18,7 do 19,2 kg/dan odn. 0,5 kg (34), što je niže u odnosu na povećanje dobijeno u ovom istraživanju. Naši rezultati su takođe veći u odnosu na rezultate (35,36) gde je zabeleženo neznatno povećanje prinosa mleka (0,1-0,5 kg). Dok u radovima (37,38) nije bilo značajnijih promena u proizvodnji mleka izloženih krava, u ispitivanjima (39,40) prinos mleka je bio neznatno smanjen (za 0,16 kg) u toku perioda izloženosti naizmenične struje od 4 mA (60 Hz), ali ne i statistički značajno (p>0,05).

Tabela 4. Proizvodnja mleka kod krava u oglednoj i kontrolnoj grupi

Nedelja	Grupa	Grla	$\bar{x} \pm \bar{S}x$		SD	CV%	min.- maks.	Razli ka
Pripremna	*ogled.*	210	31,50	0,32	4,63	14,71	15,5-43,4	0,67^{NS}
	kontr.	210	30,83	0,31	4,43	14,37	20,2-43,6	
UKUPNO	*ogled.*	1.260	31,10	0,29	4,77	15,33	14,7-59,0	2.09**
	kontr.	1.260	29,01	0,31	4,99	17,20	11,3-43,6	

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PREDNOSTI ELEKTROMAGNETNE STIMULACIJE I NJENE INTERAKCIJE SA BIOSTRUJAMA U PROIZVODNJI MLEKA

Na osnovu svega navedenog, integrišući fiziološka znanja i znanja dobijena iz ekperimenta zaključujemo da postoje određene prednosti ovakvog vida stimulacije. Metod je konforan i ne remeti dnevni ritam životinja, praktično izostaju neprijatnosti. Naprotiv, uz muziku Baha i Mocarta, uočeno je da su životinje opuštenije, ležernije i u većoj međusobnoj harmoniji. Za razliku od ogledne grupe, životinje u kontrolnoj grupi su bile nekompaktne i uznemirenije, posebno u večernjim satima. Postupak je neinvazivan i bezbolan, moguća je primenljivost u farmskim uslovima držanja životinja, pri čemi nije ugrožena niti umanjena njihova produktivna sposobnost, a uz adekvatne uslove ishrane i nege. Postiže se veći ekonomski efekat, jer uz isti utrošak hrane, dobija se veći prinos mleka po grlu. Dobijeni razultati u ovom radu, nedvosmisleno potvrđuju značaj biofizičkog delovanja na organizam životinja. Primenom EM stimulacije proizvodnja mleka se može značajno povećati, bez narušavanja prirodnog balansa, pri čemu hemijski sastav mleka ostaje nepromenjen.

REFERENCE

1. *Jokela K. Ionisoimaton säteily ja sähkömagneettiset kentät. In: Sähkömagneettiset kentät. Nyberg H, Jokela K (eds.) Säteily- ja ydinturvallisuus - sarja, osa 6. Karisto, Hämeenlinna pp. 11-25, 2006.*
2. *Wiltschko R., Wiltschko W. Magnetoreception. BioEssays 2006, 28, 2, 157-168.*
3. *Belova N., Avalos-Acosta D. The effect extremely low frekquency alternating Magnetic fields on the behaviour of animals in the presence of the Geomagnetic fields. Journal of biophysics 2015, 2015, 423838.*
4. *Cifra M., Fields J.Z., Farhadi A. Electromagnetic cellular interaction. Prog.Biophys.Mol.Biol. 2011, 105, 223-246, doi: 10.1016/j.pbiomolbio.2010.07.003.*
5. *Havelka D., Cifra M. Calculation of the electromagnetic fields around a mitochondria. Acta Polytechnia 2009, 49, 58-63.*
6. *Havelka D., Cifra M., Kuĉera O., Pokorny J., Vrba J. High-frequency electric fields and radiation characteristics of cellular microtubule network. J.Theor.Biol. 2011, 286, 31-40.*
7. *Gu J., Firestein B.L., Zheng J.Q. Microtubles in dendritic spine development. J.Neurosci. 2008, 28, 12120-12124.*
8. *Tuszynski J.A., Hameroff S., Satarics M.V., Trpisove B., Nip L.A. Ferroelectric behavior in microtubule dipole lattices: implications for information processing, signalling and assembly/disassembly. J.Theor.Biol. 1995, 174, 371-380.*
9. *Hideg E., Kobayashi M., Inaba H. Spontaneous ultraweak light emmision from respiring spinach leaf mitochondria. Biochemi. Biophys.Acta (BBA)- Bioenerg., 1991,1098, 27-31.*
10. *Glade N.,Tibony J. Breaf exposure to high magnetic fields detemines microtubules self-organisation by reaction-diffusion processes. Biophys.Chem. 2005, 115, 29-35.*
11. *Wiltschko R., Wiltschko W. Magnetic orientation of animals. Springer, Berlin, str. 27-41, 19995.*
12. *Wiltschko W., Wiltschko R. Magnetic orientation in birds, Journal of experimental Biology 1996, 199, 1, 29-38.*
13. *Raković D. Osnovi biofizike, IASC & IEFPG Beograd, str.244, 2008.*
14. *Tenforde T.S., Kaune W.T. Interaction of extremely low frequency electric and magnetic fields with humans. Health. Phys. 1987, 53(6): 585-606.*
15. *Crowley W.R. Neuroendocrine regulation of lactation and milk production, Compl. Physiol. 2015, 5(1), 255-291.*
16. *Bruckmaier R.M. Normal and disturbed milk ejection in dairy cows, Domestic Animal Endocrinology 2005, 29: 268-273.*
17. *Svennersten S.K., Olsson K. Endocrinology of milk production, Domestic Animal endocrinology 2005, 29 (2): 241-258.*
18. *Reece O.W., Erickson H.H., Golf P.J., Vemura E.E. Duke's Physiology Domestic Animals, 13th edition, by John Wiley&Sons,Inc, 2015.*
19. *Đoković R., Cincović M., Belić B. Fiziologija i patofiziologija metabolizma krava u peripartalnom periodu, Poljoprivredni fakultet, Novi Sad, str. 25-28, 2014.*
20. *Milankov Z. Efekat aure, Novi Sad, str. 7-10, 2015.*
21. *Baureus K.C.L., Sommarin M., Persson B.R., Salford L.G., Eberhardt J.L. Interaction between weak low frequency magnetic fields and cell membranes, Bioelectromagnetics 2003, 24(6): 395-402.*
22. *Foster K.R. Mechanism of interaction of extremely low frequency electric fields and biological systems, Radiat.Prot.Dosimetry 2003,106(4): 301-310.*

ISBN 978-86-7520-611-8

23. *Lucietta B., Grazia T., Fabio F., Michela Z., Pietro M., Maurizio B., Francesco B. Weak static and extremely low frequency magnetic fields affect in vitro pollen germination, The Scientific World Journal 2011, 11-875-890.*
24. *Panagopoulos D.J., Karabarbounis A., Margaritis L.H. Mechanism for sction of electromagnetic fields on cells, Biochem.Biophys.Res.Commun. 2002, 298(1): 95-102.*
25. *Lefcourt A., Akers R.M. Endocrine responses of cows subjected to controlled voltages during milking. J. Dairy Sci. 1982, 65:2125-2130.*
26. *Kaneko J.J., Harvey W.J., Bruss L.M. Clinical Biochemistry of Domestic Animals, Body fluid compartments, Six edition, Academic Press, str.531, 1989.*
27. *Jazbec I. Kliniĉko laboratorijska dijagnostika, Veterinarska fakulteta, Ljubljana, 1990.*
28. *Jovanović M. Fiziologija domaćih životinja, Beograd-Zagreb, 1988.*
29. *Burchard J.F., Nguyen D.H., Block E. Macro and trace element concentrations in blood plasma and cerebrospinal fluid of dairy cows exposed to electric and magnetic fields. Bioelectromagnetics 1999b, 20:358–364.*
30. *Shivers R.R., Kavaliers M., Teskey G.C., Prato F.S., Pelletier R.M. Magnetic resonance imaging temporarily alters blood barrier permeability in the rat. Neurosci. Lett. 1987, 776:21-31.*
31. *Barbier E., Dufy B., Veyret B. Stimulation of Ca^{2++} influx in rat pituitary cells under exposure to a 50 Hz magnetic fields. Bioelectromagnetics 1996, 17(4):303- 311.*
32. *Li K., Ma S., Ren D., Li Y., Ding G., Liu J., Guo Y., Gio G. Effect of Electromagnetic Pulse in Serum element levels in rat, Biological trace Element Research 2014, 158, 1, 81-86.*
33. *Bernardini C., Zannoni A., Turba M.E., Bacci M.L., Forni M., Mesirca P., Remondini D., Castellani G., Bersani F. Effect of 50 Hz sinusoidal magnetic fields on Hsp27, Hsp70, Hsp90 expression in porcine aortic endothelial cells (PAEC). Bioelectromagnetics 2007, 28(3): 231-237.*
34. *Rodriguez M., Petitclerc D., Burchard J., Nguyen D.H., Block E. Blood melatonin and prolactin concentrations in dairy cows exposed to 60 Hz electric and magnetic fields during 8 h photoperiod. Bioelectromagnetics 2004, 25:508–515.*
35. *Aneshansley D.J., Gorewit R.C., Price L.R. Cow sensitivity to electricity during milking. J.Dairy Sci. 1992, 75(10): 2733-2741.*
36. *Aneshansley D.J., Price L.R. Effect of voltages on cows over a complete lactation: 1. Milk yield and composition. J.Dairy Sci. 1992, 75(10): 2719-2725*
37. *Reinemann D.J., Stetson L.E., Laughlin N.K. Water, feed, and milk production response of dairy cattle exposed to transient currents. ASAE, Annual International Meeting Technical Paper no. 95-3276, 1996.*
38. *Reinemann D.J., Stetson L.E., Laughlin N.K. Water, feed, and milk produc, tion response of dairy cattle exposed to transient currents.Trans. ASABE 2005, 48(1): 385-392.*
39. *Gorewit R.C., Aneshansley D.J., Price L.R. Effect of voltages on cows over a complete lactation: 2. Health and reproduction. J. Dairy Sci. 1992, 75(10): 2726- 2732.*
40. *Gorewit R.C., Scott N.R., Czarniecki C.S. Response of dairy cows to alternating electrical current administered semi randomly in a non-avoidance environment. J.Dairy Sci. 1985, 68(3): 718-725.*

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Review
Pregledni rad

EU PROJECT ON ANIMAL RESCUE FROM FLOOD IN TURKIYE

PROJEKAT EU O SPASAVANJU ŽIVOTINJA OD POPLAVA U TURSKOJ

Halil Selcuk BİRİCİK[a]*

[a]Afyon Kocatepe University, Faculty of Veterinary Medicine- Afyonkarahisar-Türkiye
**Corresponding author: hsbiricik1@gmail.com*

ABSTRACT

Under the scope of Mitigating Flood Risk in flooded areas in the Southeastern Anatolian Region of Türkiye, the project titled as “Capacity Building for Decreasing Animal Losses from Flood in Sanliurfa” has been carried out by Sanliurfa City Disaster and Emergency Management Directorate, Southeastern Anatolia Region Culture, Research and Development Association and Agricultural Development Cooperative. The objectives were to improve the capacities of participant organisations about animal welfare in flood management and in the prevention of flood. Veterinarians, emergency personnel and geographers educated volunteer young people to establish animal rescue teams. International Animal Rescue Conference and Animal Rescue Event have been carried out in Sanliurfa jointly with Royal Veterinary college and Hampshire Municipality fire department.

Key words: Animal rescue, flood, Türkiye.

SAŽETAK

U okviru Ublažavanja rizika od poplava u poplavljenim područjima u jugoistočnoj Anadoliji u Turskoj, projekat pod nazivom „Izgradnja kapaciteta za smanjenje gubitaka životinja od poplava u Sanliurfi“ je sprovela Direkcija za katastrofe i vanredne situacije grada Sanliurfa, Jugoistočni region Anatolija. Udruženje za kulturu, istraživanje i razvoj i Poljoprivredna razvojna zadruga. Ciljevi su bili unapređenje kapaciteta organizacija učesnica o dobrobiti životinja u upravljanju poplavama iu prevenciji poplava. Veterinari, osoblje hitne pomoći i geografi edukovali su mlade volontere da osnuju timove za spasavanje životinja. Međunarodna konferencija o spašavanju životinja i događaj spašavanja životinja održani su u Sanliurfi zajedno sa Kraljevskim veterinarskim koledžom i vatrogasnom službom opštine Hampshire.

Ključne reči: spasavanje životinja, poplava, Turska.

INTRODUCTION

Within the scope of animal welfare, which has gained great importance for nearly fifty years, it is seen that significant progress has been made in developed countries in "Safe Animal Rescue" in Veterinary medicine practice (1-5).

Mitigating Flood Risk in Flooded Areas in the Southeastern Anatolia Region (SAR) project started within the scope of the European Union Pre-accession Financing Agreement in 2006. The project was jointly financed by the European Union and Republic of Türkiye. The Contracting Authority for grant contract with Grant Beneficiaries was the Central Finance and Contracts Unit (CFCU).

Heavy rains have negatively affected the Sanliurfa region socially, environmentally and economically. The flood caused people’s deaths (n= 42). Many animals destroyed, hundreds of houses and dairy seriously damaged and wide agricultural areas were negatively influenced. The first assessments have shown that insufficient infrastructure and rapid growth of unplanned settlements under flood risk are factors determining the influence of flood. Regarding with Mitigating Flood Risk in flooded areas in the SAR Project open call, Sanliurfa Disaster and Emergency Management

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Directorate and SAR Culture, Research and Development Association prepared project titled as “Capacity Building for Decreasing Animal Losses from Flood in Sanliurfa” and accepted by EU Union.

Main objectives were to prevent flooding and its effects in the long term through local institutional capacity building in the SAR. Specific objectives were to improve the capacities of local governmental and non-governmental organisations in flood management and in the prevention of flood, mitigate infrastructure, economic and social losses caused by flood. To reach the project objectives in the SAR provinces, social support and physical planning/investment grant schemes have been launched.

Final beneficiaries were small-scale families working with animal husbandry in Sanliurfa Center, Harran and Akcakale districts; large-scale animal enterprises producing meat and milk; race horse and working horse and pet owners. Target Groups were young people dealing with animal husbandry in the region; small and large ruminants, horses, poultry, pet animals; local people: animal owners and people under risk of contagious diseases from animal wasted in flooding.

ACTIVITIES

Activity 1: Establishment of Project Team

Mahmut Sönmez, director of Sanliurfa Disaster and Emergency Management presidency; Profs Gürbüz Aksoy and Halil Selçuk Biricik, Faculty of Veterinary Medicine members have been assigned as contact person, coordinator and technical assistant during the project

Activity 2: Preparation of Training Materials

Training materials have been prepared by the project team. Presentations were about first aid in small and large animals, rescuing animals in dramatic situations with appropriate methods, infectious diseases, proper techniques of animal handling, restraint and evacuation, hygiene, post flood animal care and nutrition.

Activity 3: Training

It has been planned to train that volunteer young people (n=70) dealing with animal husbandry to establish animal rescue teams. Animal welfare training duration was ten days. Trainers were from veterinarians, Sanliurfa emergency personnel and geographers. It has been emphasized to participants that rescuing animals is important besides people in floods or other natural disasters. Some part of training has been carried out by emergency search and rescue personnel. In this context, rope application for animals has been shown practically. In addition, duties of officials, fire protection, humanitarian rescue in the earthquake and flood has been explained.

Fig. 1-2: International Animal Rescue Conference

DISCUSSION

Animal losses occur because animals aggrieved in disaster are not saved in time and rescued with proper techniques. Sometimes it has been encountered by inappropriate animal intervention news. If emergency personnel have enough experience besides full rescue equipment, society would not encountered with undesired outcomes.

Hampshire municipality, fire department in UK and London Royal Veterinary College works together on animal welfare. Prof. Dr. Josh Slater, equine hospital director, Royal Vet. College and Jim Green, firefighter in Hampshire fire and rescue service, continue this collaboration with several conferences. We contacted with them for the purpose of the exchange information on animal welfare, invited to the the International Animal Rescue Conference. The conference has been held in Sair Nabi Culture Center, Sanliurfa jointly with Royal Vet. College and Hampshire Municipality. Jim Greeen and Josh Slater, recipient of the British Equine Veterinary Association's welfare award 2019, joined the conference as invited speakers and presented about modern animal rescue techniques (Fig. 1-2). Following the conference, "Animal Rescue Event" to show practical aid techniques with equipments has been performed by participation of foreign experts, disaster and emergency personnels, fire crews, trained young volunteers, Veterinarians working private or depending on agricultural directorate and farmers.

With the activities under the project, emergency personnels gained experience about animal welfare. Animal welfare training has been given to 70 young people engaged in animal husbandry. These volunteers have became aware of intervening animals together with veterinarians and emergency personnels using appropriate techniques. Since they have information about animal rescue in the potential disasters and animal casualties, economic losses will be reduced. Also after experience about proper care and feeding of animals, their income derived from animals will possibly increase.

Here are the opinions of several trained volunteers: " I learned how much importance was given to animals with this project. Humans without animal love can not give any benefit to human being." Omer Demirkol; "We've learned a lot in this short time." Celal Şen ; " Animal rescue is the first project I participated and this has increased my animal love and I became a little more sensitive towards animals." Ibrahim Felhan; "This project taught me very important points both financially and morally in my life." Cuma Doğan; "How happy if we could save an animal." I. Halil Tuz.

REFERENCES

1. *Fürst A., Keller R., von Salis B. Entwicklung eines verbesserten Haengegeschirrs für Pferde: Das TierBergungs- und Transportnetz (TBTN). Pferdeheilkunde 2006, 22: 67-772.*
2. *Fürst A., Keller R., Kummer M., Manera C., von Salis B., Auer J., Bettschart R. Evaluation of a new full-body animal rescue and transportation sling in horses. Journal of Veterinary Emergency and Critical Care 2008, 18: 619-625.*
3. *Kenel L., Keller R., VonSalis B., Fürst A. Entwicklung des Grosstier-Vertikalbergungsstets (GTVBS)- eine Weiterentwicklung des TBTN (Tier-Bergungs-und Transportnetz). Pferdeheilkunde 2016, 32: 141-147.*
4. *Sabine Kaestner R. How to manage recovery from anaesthesia in the horse-to assist or not to assist. Pferdeheilkunde 2010, 26: 604-608.*
5. *Von Salis B., Keller R., Fürst A. Die Entwicklung des Grosstier-*Rettungsdienstes Scweiz und Liechtenstein. *Hundkatzepferd 2008, 6: 44-47.*

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Article
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INFRACRVENA TERMOGRAFIJA – VARIJABILNOSTI I KORELACIJE IZMERENIH TEMPERATURA KOD KRAVA

INFRARED THERMOGRAPHY - VARIABILITY AND CORRELATIONS OF MEASURED TEMPERATURES IN COWS

Bojan Blond[a*], Mira Majkić[a], Jovan Spasojević[a], Miodrag Radinović[a], Jovan Stanojević[a]

[a] ALDAHRA Serbia, Beograd
[b] Department of Veterinary medicine, Faculty of Agriculture, University of Novi Sad, Novi Sad, Trg Dositeja Obradovića 8, Republic of Serbia
*Corresponding author: bojan.blond@gmail.com

M.M. https://orcid.org/0009-0007-0110-7327, J.Sp. https://orcid.org/0000-0001-5654-5796, M.R. https://orcid.org/0000-0002-4390-4670 , J.St. https://orcid.org/0009-0000-3328-6963

SAŽETAK

Upotreba termovizijske kamere predstavlja pouzdanu, neinvazivnu metodu procene tolpotnog stresa. Emisivnost koju površina tela odaje se kod goveda kreće od 0,93-0,98, što infracrvenu termografiju čini izuzetno prikaldnom metodom. Merenjem temperature različitih anatomskih regija može se odrediti toplotna opterećenost kao i njihova međusobna varijabilnost u odnosu na spoljašnje temperature. Termovizijskom kamerom je moguće napraviti maksimalne, srednje i minimalne IRT (infrared termograme).Takođe je moguće proceniti koji termogrami su najprecizniji za procenu toplotnog opterećenja. Korelacione analize na termogramima pokazuju značajnu povezanost između spoljašnje ambijentalne temperature i temperature različitih regija tela kod krava u toplotnom stresu. Koeficijent determinacije je u proseku iznostio 94,27%, što ukazuje na veoma visoku vezu između temperature površine tela i ambijentalne temperature. U našem eksperimentu bavili smo se posebno temperaturama abdomena i temperature regije glave. Prosečne, maksimalne i pozicione vrednosti temperature ove dve regije međusobno značajno koreliraju tako da je koeficijent determinacije od 96,33% za proseke, do preko 98% kada su u pitanju maksimalne vrednosti temperature date regije ili pozicione vrednosti piksela po uzdužnim osama regije. Sledeća značajna regija je regija vimena, a nađena je značajna korelacija između maksimalne temperature vimena sa maksimalnim temperaturama regije glave (koeficijent determinacije 93%) i regije abdomena (koeficijent determinacije 98%). Regija ekstremiteta ne pokazuje značajne korelacije sa regijom glave, dok je korelacija sa abdomenom bila na nivou 82%. I na kraju, postojala je značajna povezanost između temperature leve i desne strane tela sa koeficijentom determinacije od 97%. Sve navedeno potvrđuje da je pomoću podataka poreklom sa termograma moguće odrediti toplotnu opterećenost kod krava. U ovom radu je dat i detaljan pregled dosadašnjih različitih korelacionih istraživanja kod infracrvenog merenja.

Ključne reči: infracrvena termografija, toplotni stres, termogram, korelacije.

ABSTRACT

The use of a thermal imaging camera is a reliable, non-invasive method of assessing compressive stress. The emissivity given by the body surface in cattle ranges from 0.93-0.98, which makes infrared thermography an extremely suitable method. By measuring the temperature of different anatomical regions, it is possible to determine the thermal load as well as their mutual variability in relation to external temperatures. With the thermal imaging camera, it is possible to make maximum, medium and minimum IRT (infrared thermograms). It is also possible to evaluate which thermograms are the most accurate for assessing heat load. Correlation analyzes on thermograms show a significant relationship between the external ambient

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temperature and the temperature of different body regions in cows under heat stress. The coefficient of determination averaged 94.27%, indicating a very high correlation between body surface temperature and ambient temperature. In our experiment, we dealt specifically with the temperatures of the abdomen and the temperature of the head region. The average, maximum and positional temperature values of these two regions correlate significantly with each other so that the coefficient of determination is 96.33% for averages, up to over 98% when it comes to the maximum temperature values of a given region or the positional values of pixels along the longitudinal axes of the region. The next important region is the udder region, and a significant correlation was found between the maximum temperature of the udder with the maximum temperatures of the head region (determination coefficient 93%) and the abdomen region (determination coefficient 98%). The limb region does not show significant correlations with the head region, while the correlation with the abdomen was at the level of 82%. And finally, there was a significant correlation between the temperature of the left and right sides of the body with a coefficient of determination of 97%. All of the above confirms that it is possible to determine heat stress in cows using data from thermograms. In this paper, a detailed overview of various correlational researches in infrared measurement is given.

Key words: infrared thermography, thermal stress, thermogram, correlations.

KORELACIONA ISTRAŽIVANJA I VARIRANJE PARAMETARA SA INFRACRVENIH TZERMOGRAMA

Upotreba termovizijske kamere predstavlja pouzdanu, neinvazivnu metodu procene tolpotnog stresa. Emisivnost koju površina tela odaje se kod goveda kreće od 0,93-0,98, što infracrvenu termografiju čini izuzetno prikaldnom metodom. Merenjem temperature različitih anatomskih regija može se odrediti toplotna opterećenost kao i njihova međusobna varijabilnost u odnosu na spoljašnje temperature. Termovizijskom kamerom je moguće napraviti maksimalne, srednje i minimalne IRT (infrared termograme).Takođe je moguće proceniti koji termogrami su najprecizniji za procenu toplotnog opterećenja. Na temperature srednjih ili minimalnih IRT mogu uticati spoljašnji faktori kao što su adhezija kapljica vode, blata ili fecesa na površini kože, pa mogu dovesti do lokalnog smanjenja temperature.

Studija Uddin et al., 2020 (1) je imala za cilj da ispita promene u temperaturama pojedinih regija tela (regija oka i koronarna regija prednjih ekstremiteta) u odnosu na spoljašnje temperature. Pravljeno je nekoliko termograma (2 termograma za regiju oka kod 10-12 krava i 2 termograma za koronarnu regju prednjeg ekstremiteta kod 14-16 krava) kako bi se otkrile varijacije u temperaturi površina koje se snimaju Izračunate su maksimalne izmerene temperature površine regija kao i minimalne i prosečne vrednosti. Rezultati su pokaali da su maksimalno izmerene površine bile manje promenljive od minimalnih ili prosečnih vrednosti,. Nekoliko autora (2,3) je takođe identifikovalo da maksimalne IRT temperature spoljašnjih površina tela bolje detektuje prisustvo lezija, infekcija ili stresa, pa se ovakvi termogrami mogu koristiti I za detekciju toplotnog opetercénja. Takođe je nađena pozitivna korelacija između lateralne površine vimena, kvaliteta mleka i spoljašnje temperarure (4). Uddin et al.,2020 (1) su došli do zaključka da je izmerena temperatura očne regije manje promenljiva u odnosu na temperaturu ekstremiteta. Razlog za ovakve rezultate može se objasniti činjenicom da temperstura određenih regija tela zavisi od lokalnog metabolizma protoka krvi i emisivnosti toplote. Visoka perfuzija krvi se javlja u delovima kože bez dlake i mekim tkivima kao što je oko, pa se ove regije smatraju dobrim pokazateljima toplotnog opterećenja i nisu toliko promenljive u zavisnosti od promena u spoljašnjoj temperaturi. Pri konstantno visokim spoljašnjim temperaturama, ova regija neće pokazivati veće varijabilnosti u temperature. Shodno tome temperatura oka, baze uha i vimena se smatraju manje varijabilnim od drugih delova tela. Smatra se da se maksimalni IRT oka poklapa sa stalnom telesnom temperaturom tela koja obično malo fluktuira jer životinje mogu da održavaju svoju telesnu temperaturu u relativno uskom opsegu uključivanjem kompenzatornih mehanizama. Chruch et al., 2014 (5) su primetili da je pri direktnom izlaganju sunčevoj svetlosti došlo do povećanja temperature oka za 0,56°C. Temperatura regije oka zbog svojih anatomskih specifičnosti reaguje na povećanje ambijentalnih temperatura, ali kada dodje do naglog povećanja ambijentalne temperature. U slučajevima kada je ambijentalna temperatura konstantno visoka ta regija, ne pokazuje veliku varijabilnost u odnosu na druge regije tela.

U istraživanju Darlene Dos Santors Daltro et al., 2017 (6) je rađena procena toplotnog stresa korišćenjem termovizijske kamere kao neinvazivne procedure Rađene su korelacione analize između različitih regija na telu krava. Rezultati su pokazali da postoji pozitivna korelacija (niska do visoka) izrmđu regije oka, desne polovine tela, lateralne strane vimena kao i zadnje strane vimena. Regresione analize su pokazale da se regija vimena smatra optimalnom za procenu toplotnog stresa kod krava, i smatra se da su promene u maksimalnom IRT bolje povezane sa stresnom reakcijom i metaboličkim odgovorom. Razlog za ovakve rezultate se može objasniti bogatom vaskularnom mrežom u regiji vimena i intenzivnim tkivnim metabolizmom.

U istraživanju Vadav et al., 2017 (7) nađeno je da pojedine regije n telu menjaju tempersturu u zavisnosti od temperature spoljašnje sredine. Pri spoljašnjoj temperaturi od 35 i 40° C najtoplije regije su bile lumbalna regija, regija vrata i regija butina, i regija zadnjih ekstramiteta. Posmatrajući navedene regije primećena je niža varijacija između regije vrata, lumablnog dela i regije butina, dok je veća varijabilnost primećena u regiji zadnjih ekstremiteta. Razlog za ovakve rezultate se može pripisati rzličitim kapacitetom tkiva za eliminaciju viška toplote. De Lima et al., 2013 (8). Church et al., 2014. (5) su primetili da je temperatura vimena, nosa i ušiju u pozitivnoj korelaciju sa spoljašnjim temperaturama. Chruch et al., 2014 (5) su došli do zaključka da solarna radijacija ima uticaja na spoljašnju temperaturu pojedinih regija. Takođe je primećeno da krave sa pretežno crnom bojom krzna imaju veće spoljašnje temperature kože, kada su direktno izložene sunčevoj svetlosti.

Istraživanje Vadav et al., 2017 (7) su pokazali sledeće rezultate: Na temperaturi od 35° C temperatura regije glave je bila 35,94 ° C dok je na temperaturi od 40°C iznosila 40,39°C Temperatura regije vrata je pri spoljašnjoj temperaturi od 35°C iznosila 36,94°C, a pri temperaturi od 40°C je iznosila 40,74° C. Temperatura lumbalne regije je pri spoljašnjoj temperaturi od 35°C iznosila 37,01°C, a pri temperaturi od 40°C je iznosila 40,83°C. Regija butine je pri spoljašnjoj temperaturi od 35° C iznosila 36,58°C, a pri temperaturi od 40°C je iznosila 40,17°C. Temperatura regije zadnje noge je pri spoljašnjoj temperaturi od 35°C iznosila 35,21°C, dok je pri temperaturi od 40°C iznosila 38,69°C Korelacione analize su pokazale da postoji visoka korelaciona veza između pomenutih regija i spolajšnje temperature (0.78-0,92).

Marcelo Daniel Ghezzi et al., 2023 (9) je imala za cilj da ispita uticaj spoljašnjih temperatura na temperature pojedinih regija kod krava. Rezultati su pokazali da jutarnje merenje temperatura regije oka i periokularnog regiona ima maksimalnu vrednost od 36,8°C, dok su srednja i minimalna temperatura 35,5 i 35°C. Poredeći podnevna merenja sa jutarnjim može se zakljkučiti da je maksimalna temperatura porasla za 0,3°C, da je srednja temperatura porasla za 0,2°C, a minimalna temperatura je smanjena za 0,6°C.

U istraživanju Montaholi et al. (10) je ispitana efikasnost korišćenja termovizijske kamere u proceni toplotnog stresa kod goveda Životinje su snimane u jutarnjim i popodnevnim časovima. Napravljeni su IRT snimci različitih delova tela. Rezultsti su pokazali da je glutealna regija pokazala najveću fluktuaciju u odnosu na ostale delove tela, ali razlika između levog i desnog glutealnog područja nije bila značajna. Razlika je bila 0,1°C. Promene temperature zabeležene su i na regiji trupa, ali je regija trupa imla niže temeperature u odnosu na glutealnu regiju. Regija papaka je takođe pokazala dnevne fluktuacije. U jtarnjim časovima temperatura papaka je iznosila 28,8°C, a u popodnevnim časovima je izmerena temperatura od 31,5°C.

Uočene su umerene do visoke korelacije (0,58–0,88). između temperature površine kože i ambijentalnih temeperatura. Zadnji deo i temperatura levog boka imale su nižu korelaciju (0,58 i 0,62). Temperature prednjeg dela papaka bile su u velikoj korelaciji sa visokim ambijentalnim temperaturama (levo: 0,83; desno: 0,88). Temperature desnog boka i gluteusa su imale niže korelacije u poređenju sa temperaturom papaka. Temperaturna razlika između levog i desnog boka zajedno sa temperaturama celog tela i trupa imale su srednje vrednosti korelacije sa ambijentalnim temperaturama. Levi bok 0,62, desni bok 0,72 celo telo 0,66, trup 0,66

Isti autori su predložili tzv. Termoprozore. Termoprozori predstavljaju regije na telu koje su najosetljivije na promene spoljašnje temperature Predloženi termo prozori bi mogli biti: Aurikularni. Ovaj prozor indirektno beleži zračenu temperaturu bubne opne, koju snabdevaju aretrije auricularis medialis i marginalne arterije. Drugi termalni prozor bi mogao biti očni. Ovaj region uzima u obzir temperaturu koja dolazi iz očne jabučice i očnih

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kapaka Oba regiona se snadbevaju krvlju koja potiče iz supraorbitalne vene i arterije, kao i granama oftalmične arterije. Treći termalni prozor bi moga da bude nazalni. Ovaj prozor procenjuje promene u temperaturi izdahnutog vazduha, uz vaskularizaciju površnih kapilara maksilarne arterije. Sledeći termalni prozor bi mogao da bude lakrimalni karunkul. Ovaj region, poput očnog termalnog prozora, odražava cirkulaciju krvnih kapilara maksilarnih i infraorbitalnih arterija, zajedno sa simpatičkom inervacijom u medijalnoj komisuri očnih kapaka.

Do sličnih rezultata su došli Bakony et al. (11) u novijim istraživanjima. Reultati su pokazali da postoje određene regije koje mogu biti indikator toplotnog opterećenja. Rezultati su pokazali da na osnovu korelacionih analiza regija njuške, regija ušiju, predeo lopatice i regija nogu pokazuju najveće fluktuacije u najtoplijem delu dana. Regija njuške je područje bez dlake i obično je vlažna, čime se promoviše gubitak topkote isparavanjem, i time se postiže veća razlika između medijuma i okoline. Rezultati su pokazali da kada postoji mala razlika između temperature tela i ambijentalne temperature, dijagram rasipanja temperatura pojedinih regija ima tendenciju da pokazuje manju varijabilnost. Rezultati istraživanja su pokazali da većina regija koje su snimane imaju temperature od 35°C. Ipak najjače korelacije su primećene u predelu vrta, uha iskapule I ekstremiteta (0,78-0,83).

Rezultati istraživanja Martello Luciane Silva et al. (12) su pokazali da u najtoplijrm delu dana najmanju varijabilnost pokazuje regija oka. Merenje je radjeno pri termoneutralnim uslovima i u najtoplijem delu dana. Regija trupa, bokova i glitealne regije su bili regioni sa srednjim vrednostima IRT-a i većom varijabilnošću u temperaturi, dok je regija papaka predstavljena kao najhladnija. Temperatura pojedinih anatomskih regija zavisi od količine krvi koja protekne kroz dato područje, pa će područja koja imaju bolju vaskularizaciju i metaboličku aktivnost biti pod većim uticajem promena u ambujentalnoj temperturi. U regiji glave se nalaze centar za regulaciju telesne temperature, pa se niska varijabilnost tempeatura u ovoj regiji tokom najtoplijeg dela dana može objasniti time da mreža krvnih sudova i termoregulacioni centar reaguju na promene u spojašnjim temperaturama, ali kada su spoljašnje temperature konstantno visoke, Zato se temperatura u regiji glave može smatrati dobrim indikatorom temperature izmerne per recti. Regija glave se može smtarati dobrim indikatorom tpolotnogs stresa. Rezultati istraživanja Peng et al. (13) Su pokazali da je u najtoplijem delu dana čeona regija pokazivala više temperature. Isto istraživanje je pokazalo povišene temperatzre u regiji vimena, ali su one bile niže od temperatura koj su izmerene u regiji glave. Razloga za lokalno povećanje regije vimena može se objasniti time da regija vimena ima tanki kožu bez gustog dlačnog pokrivača, kao i da je dobro vaskularizovana i metabolički aktivna. Prednja strana vimena je pokauzala veće temperature u odnosu na regiju zadnjeg dela vimena. Razlog je taj što je zadnji deo vimena prirodno “zaklonjen” zadnjim ekstremitetima.

Studija Idris et al. (14) je pokazala da Maximalne vrednosti IRT-a bolje pokazuju toplotnu opterećenost od srednjih i minimalnih vrednosti. Ista studija je pokazala da maksimalne vrednosti IRT regije oka, posebno regije oko unutrašnjih uglova očne duplje predestavlja dobar indikator toplotnog opterećenja. Očna regija se nalazi blizu hipotalamusnog centra u CNS-u, a takođe poseduje i superficijalne kapilare u lakrimalnom karunkulu I zadnjem rubu očnih kapaka. Naveden formcije su visoko inervisane simpatičkim nervnim sistemom. Oči su vaskularizovane krvnin sudovima mrežnjače koji su slični krvnim sudovima u CNS-u Zbog karakteristične anatomske građe, regija oka predstavlja dobar pokazatelj toplotnog opterećenja, I ne pokazuje veće varijabilnosti kod konstantno visokih aambijentalnih temperatura.. Regija prednjeg i zadnjeg dela tela pokazala je veću varijabilnost, pri čemu je regija u blizini srca bila toplija. Ovo se može obajsniti specifičnom mišićnom strukturom koja aktivno učestvuje u kretanju životinje i pomaže u održavanju stabilnosti, kao i obilnom vaskularizacijom koja obezbeđuje prokrvljenost posmatrane regije.

U istraživanju Salles et al. (15) je merena temperatura površine različitih regija kod krava Džersej rase u termoneutralnim uslovima. Rzultati su pokazali da je korelacija između pojedinih regoja i temperature okoline bila srednja do visoka (0,26-0,91). Visoka korelaciona veza nađena je između ambijentalne temperature i levog i desnog boka, kaudalnog dela prednje noge i čeone regije (0.85, 0.81, 0.74, 0.64 I 0.81). Visoka korelaciona veza nađena je između levog boka i čeone regije. Koerlaciona veza između temperature čeone regije i ostalih regija je bila umerena do visoka. Levi bok 0,77, levo oko 0,60, kaudalni deo leve noge 0,75. Uočena je visoka korelacija između levog i desnog boka. Rezultsti su bili očekivani pošto životine nisu hranjene neposredno pre snimanja. Smatra se da usled varenja hrane na strain gde se topografski nalazi burag dolazi do lokalnog povećanja temperature. Regija leve gladne jame se smatra dobrim indikatorom temperaturnih promena unutar buraga

Termovizijska kamera se može koristiti za procenu toplotnog opterećenja i kod svinja smatra se das su regija oka i uha dobri pokazatelji topltnog opeterećenja, ali i stresa izazvanog drugim stresogenim faktorima. Rezultati istraživanja Tucker et al., 2023 (16) su pokazali das su ove regije pokazale visoku korelaciju, ali se mora voditi računa o tome da površina koja se snima treba da bude čista. naveden regije mogu biti indikatroi stresa koji potiče i od drugih stresogenih faktora novorođena prasad se smatraju najosetljivijom kategorijom na stres, pa se očekuje da temperature navedenih regija budu više. Međutim rezultati istraživanja Tucker et al. (16) su pokazali da je temperatura navedenih regija bila viša u prvim danima nakon rođenja u odnosu na temperaturu kod tek oprašene prasadi. Razlog za ovakve rezultate može biti taj da je prasad kao polsedica pretrpljenog stresa potrošila veću količinu zaliha energije i time smanjila periferni protok krvi i lokalnu temperaturu.

Termovizijska kamera se koristi za procenu temperature tela i u humanoj medicini. Studija Švanter et al. (17) je imala za cilj da ispita ograničenja u merenju temperatura različitih regija pri određenim ambijentalnim temperaturama upotrebom termovizijske kamere. Ogled je radjen u novembru i aprilu mesecu, a merene su različite regije na licu ljudi. ambijentalne temperature nisu strogo kontroliane i iznosile su približno 20–30°C, i relativna vlažnost od 20–50%. Rezultati su pokazali da je standardna devijacija u predelu axilarne regije iznosila 36.16 ± 0.38°C. Regija lica je pokazala veću varijabilnost Standardna devijacija za regiju očiju je izmosila 0.4–0.9 °C I 0.7–0.9 °C za regiju ćela. Regija očiju je pokazala najveću varijabilnost 74% rezultata merenja se odnosilo na regiju očiju, 6,7% na regiju vrata, 9,6% na regiju usta, 2,8% na regiju čela i slepoočnice. smatra se da regija lica može biti dobar pokazatelj promena u spoljašnjim temperaturama ali u slučaju kada oči nisu pokrivene naočarima. U suprotnom može doći do smanjenja u lokalnoj temperature.

Studija Luximon et al. (18) je imala za cilj da ispita varijacije u temperature različitih regija tela kod ljudi. Rađeno je merenje regije stopala, šaka, glave i celog tela. Rezultati su pokazali da se u regiji stopala lokalna temperatura razlikje kod stopla koje je bilo prekriveno čarapama i obućom (registrovana je viša temperatura- 34°C). Temperatura pre nošenja obuće i čarapa bila je 32 Najtopliji deo je bio frontalni deo stopala. Termogram šake je pokazao opseg od 15°C kao najniži (nakon potapanja šake u hladnu vodu) i 37°C kao najviši koji je registrovan 15 min nakon efekta hlađenja. Kada se ruka potopi u hladnu vodu, dorzalna strana šake je hladnija u odnosu na volarnu. Razlika je u drugačijoj anatomskoj strukturi krvnih sudiva dorzalne strane šake. Ispitan je I uticvaj nošenja maske (hirirške i N95) na temperaturu lica. Rezultati su pokazali da je pri upotrebi hiriške maske termogram lica bila viša (29°C) u odnosu na upotrebu maske N95 gde je izmerena temperatura iznosila 27°C. Kod posmatranja termograma celog tela postoje četiri modela (PC1-4) kojima se može objasniti određeni procenat varijabilnosti PC-1 predstavlja razliku između toplog i hladnog i njime je moguće objasniti 56,86% varijacija. Drugi model je PC-2 i on predstavlja razliku u temperature leđa i ekstremiteta i ovim faktorom je moguće objasniti 12,08% ukupne varijacije. PC-3 predstavlja razlike u temperature nogu i njime je moguće objasniti 6,02% varijacije a PC-4 predstavlja razliku između nadlaktice i noge i ovim modelom je moguće objasniti 4,41% varijacija.

Studiji Kim et al. (19) je imala za cilj da utvrdi da li pojedine regije na licu mogu biti indikator telesne temperature. Regije koje su uzete u razmatranje su regija uha i regija kantusa (ugao oka gde se spajaju gornji i donji očni kapak). rezultati su pokazali da je korelacija između kornee i medijalnog kantusa bila 0,53. Temperatura timpanične regije je bila za 1-2°C viša u odnosu na tmeperaturu izmerenu termovizijskom kamerom. Temperatura medijalnog kantusa je pokazala najmanju varijabilnost uzmeđu temperatura merenih termometrom i termovizijskom kamerom.

KORELACIONA ISTRAŽIVANJA TERMOGRAMA KOD KRAVA U TOPLOTNOM STRESU NA TERITORIJI VOJVODINE TOKOM NAJTOPLIJE 2023.GODINE

Korelacione analize izvedene sun a osnovu termograma iz našeg najnovijeg istraživanja (20) pokazuju značajnu povezanost između spoljašnje ambijentalne temperature i temperature različitih

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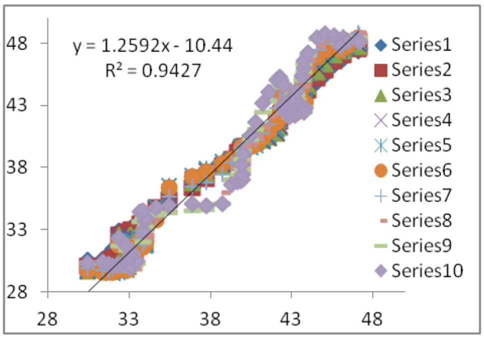
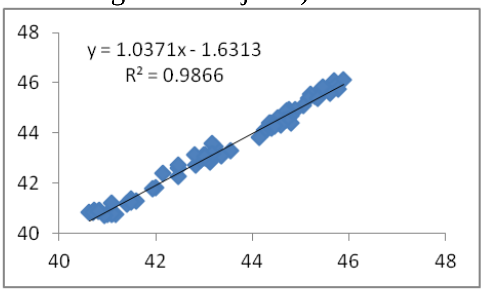
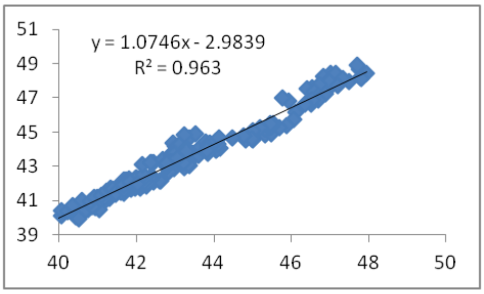
regija tela. Koeficijent determinacije je u proseku iznostio 94,27%, što ukazuje na veoma visoku vezu između temperature površine tela i ambijentalne temperature. U našem eksperimentu bavili smo se posebno temperaturama abdomena i temperature regije glave. Prosečne, maksimalne i pozicione vrednosti temperature ove dve regije međusobno značajno koreliraju tako da je koeficijent determinacije od 96,33% za proseke, do preko 98% kada su u pitanju maksimalne vrednosti temperature date regije ili pozicione vrednosti piksela po uzdužnim osama regije. Sledeća značajna regija je regija vimena, a nađena je značajna korelacija između maksimalne temperature vimena sa maksimalnim temperaturama regije glave (koeficijent determinacije 93%) i regije abdomena (koeficijent determinacije 98%). Regija ekstremiteta ne pokazuje značajne korelacije sa regijom glave, dok je korelacija sa abdomenom bila na nivou 82%. I na kraju, postojala je značajna povezanost između temperature leve i desne strane tela sa koeficijentom determinacije od 97%. Svi rezultati su prikazani na Grafikonima 1 do 8.



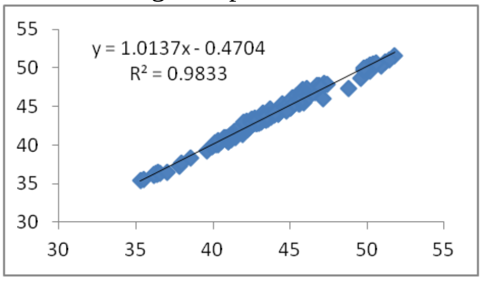
Grafikon 1. Korelacija temperature sa spoljašnjom temperaturom (temperatura tela snimane termogramom lateralno: serija 1 – abdomen, serija 2 – vime, serija 3 – prednji ekstremitet, serija 4 – zadnji ekstremitet serija 5 – ceo trup; temperatura glavene regije snimane kranijalno: serija 6 – oko, serija 7 – uho, serija 8 – nos, serija 9 – čelo, serija 10 – cela glava kranijalno).



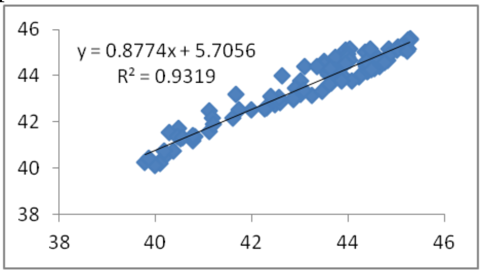
Grafikon 2. Korelacija glavenog i abdominalnog dela tela maksimalne temperature.



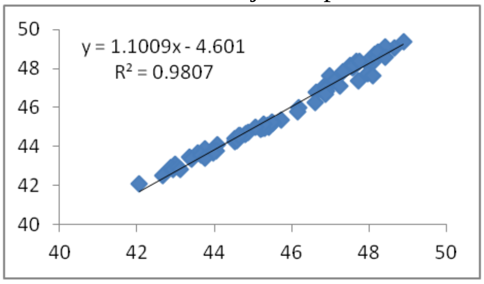
Grafikon 3. Korelacija temperature glavenog i abdominalnog dela proseci.



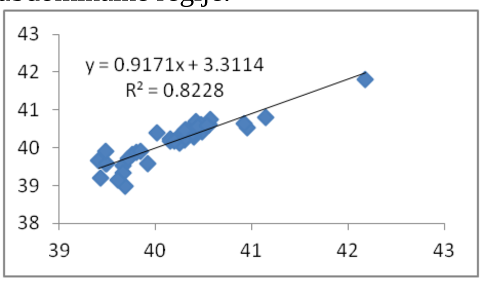
Grafikon 4. Korelacija temperature glavenog i abdominalnog dela tela odabir isto pozicioniranig piksela.



Grafikon 5. Korelacija temperature vimena i glave.



Grafikon 6. Korelacija temperature vimena i abdominalne regije.



Grafikon 7. Korelacija temperature ekstremiteta i abdominalne regije.

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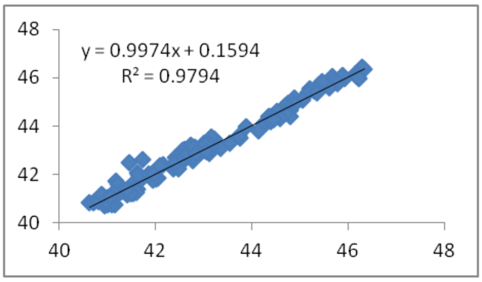

Grafikon 8. Korelacija temperature leve i desne strane tela.

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LITERATURA

1. *Uddin J., McNeill D.M., Lisle A.T., Phillips C.J. A sampling strategy for the determination of infrared temperature of relevant external body surfaces of dairy cows. International Journal of Biometeorology 2020, 64, 1583-1592*
2. *Byrne D.T., Berry D.P., Esmonde H., McHugh N. Temporal, spatial, inter-, and intra-cow repeatability of thermal imaging. J Anim Sci 2017, 95:970–979.*
3. *Byrne D.T., Berry D.P., Esmonde H., McGovern F., Creighton P., McHugh N. Infrared thermography as a tool to detect hoof lesions in sheep. TAS 2019, 3,577–588*
4. *Uddin J., Phillips C.J.C., Goma A.A., McNeill D.M. Relationships between infrared temperature and laterality. Appl Anim Behav Sci 2019, 220, 104855*
5. *Church J.S., Hegadoren P.R., Paetkau M.J., Miller C.C., Regev-Shoshani G., Schaefer A.L., Schwartzkopf-Genswein K.S. Influence of environmental factors on infrared eye temperature measurements in cattle. Res Vet Sci. 2014, 96(1),220-226. doi: 10.1016/j.rvsc.2013.11.006.*
6. *Daltro D.D.S., Fischer V., Alfonzo E.P.M., Dalcin V.C., Stumpf M.T., Kolling G.J., McManus C. Infrared thermography as a method for evaluating the heat tolerance in dairy cows. Revista Brasileira de Zootecnia 2017, 46, 374-383.*
7. *Yadav B., Singh G., Wankar A. The use of infrared skin temperature measurements for monitoring heat stress and welfare of crossbred cattle. Indian J. Dairy Sci 2017, 70(1), 1-5.*
8. *de Lima V., Piles M., Rafel O., López-Béjar M., Ramón J., Velarde A., Dalmau A. Use of infrared thermography to assess the influence of high environmental temperature on rabbits. Res Vet Sci 2013, 95(2):802–810*
9. *Ghezzi M.D., Napolitano F., Casas-Alvarado A., Hernández-Ávalos I., Domínguez-Oliva A., Olmos-Hernández A., Pereira A.M. Utilization of Infrared Thermography in Assessing Thermal Responses of Farm Animals under Heat Stress. Animals 2024, 14(4), 616.*
10. *Montanholi Y.R., Odongo N.E., Swanson K.C., Schenkel F.S., McBride B.W., Miller S.P. Application of infrared thermography as an indicator of heat and methane production and its use in the study of skin temperature in response to physiological events in dairy cattle (Bos taurus). J Therm Biol 2008, 33:468–475*
11. *Bakony M., Kovács L., Kézér L.F., Jurkovich V. The use of body surface temperatures in assessing thermal status of hutch-reared dairy calves in shaded and unshaded conditions. Frontiers in Veterinary Science 2023, 10.*
12. *Martello L.S., da Luz e Silva S., da Costa Gomes R., da Silva Corte R.R.P., Leme P.R. Infrared thermography as a tool to evaluate body surface temperature and its relationship with feed efficiency in Bos indicus cattle in tropical conditions. International journal of biometeorology 2016, 60, 173-181.*
13. *Peng D., Chen S., Li G., Chen J., Wang J., Gu X. Infrared thermography measured body surface temperature and its relationship with rectal temperature in dairy cows under different temperature-humidity indexes. International journal of biometeorology 2019, 63, 327-336.*
14. *Idris M., Uddin J., Sullivan M., McNeill D.M., Phillips C.J. Non-invasive physiological indicators of heat stress in cattle. Animals 2021, 11(1), 71.*
15. *Salles M.S.V., da Silva S.C., Salles F.A., Roma Jr L.C., El Faro L., Mac Lean P.A.B., Martello L.S. Mapping the body surface temperature of cattle by infrared thermography. Journal of Thermal Biology 2016, 62, 63-69.*
16. *Tucker B.S., Jorquera-Chavez M., Petrovski K.R., Craig J.R., Morrison R.S., Smits R.J., Kirkwood R.N. Comparing surface temperature locations with rectal temperature in neonatal piglets under production conditions. Journal of Applied Animal Research 2023, 51(1), 212-219.*
17. *Švantner M., Lang V., Skála J., Kohlschütter T., Honner M., Muzika L., Kosová E. Statistical study on human temperature measurement by infrared thermography. Sensors 2022, 22(21), 8395.*
18. *Luximon A., Chao H., Goonetilleke R.S., Luximon Y. Theory and applications of InfraRed and thermal image analysis in ergonomics research. Frontiers in Computer Science 2022, 4, 990290.*
19. *Kim D.W., Zhang H.Y., Yoo J.H., Park Y.S., Song H.J., Yang K.H. The correlation between tympanic membrane temperature and specific region of face temperature. Quantitative InfraRed Thermography Journal 2019, 16(1), 1-7.*
20. *Blond B., Majkić M., Spasojević J., Hristov S., Radinović M., Nikolić S., ... Cincović, M. Influence of Heat Stress on Body Surface Temperature and Blood Metabolic, Endocrine, and Inflammatory Parameters and Their Correlation in Cows. Metabolites 2024, 14(2), 104.*

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ANTHELMINTIC EFFICACY OF AGRO-INDUSTRIAL BY-PRODUCTS AGAINST GASTROINTESTINAL NEMATODES OF SHEEP: *IN VITRO TESTS*

ANTIHELMINTIČKA EFIKASNOST NUSPROIZVODA AGROINDUSTRIJE PROTIV GASTROINTESTINALNIH NEMATODA OVACA: *IN VITRO TESTOVI*

Antonio Bosco[1*], Alessandro Nappa[1], Giulia Capezzuto[1], Simona Lucibelli[1], Martina Nocerino[1], Luigia Di Donato[1], Vincenzo Musella[2], Alessandro Vastolo[1], Dieu donné Kiatti[1], Serena Calabrò[1], Monica I. Cutrignelli[1], Smaragda Sotiraki[3], Laura Rinaldi[1]

[1]University of Naples Federico II, Department of Veterinary Medicine and Animal Productions, Naples, Italy
[2]Department of Health Sciences, University of Catanzaro Magna Græcia, Catanzaro, Italy
[3]Veterinary Research Institute, Hellenic Agricultural Organisation ELGO-DIMITRA, Thessaloniki, Greece
*Corresponding author: antonio.bosco@unina.it

A.B. https://orcid.org/0000-0002-1266-2154, V.M. https://orcid.org/0000-0002-4448-8832, A.V. https://orcid.org/0000-0002-2284-9174, D.D.K. https://orcid.org/0009-0003-5445-717X, S.C. https://orcid.org/0000-0002-0758-1472, M.I.C. https://orcid.org/0000-0002-2493-9317, S.S. https://orcid.org/0000-0001-8357-5990, L.R. https://orcid.org/0000-0002-0975-2052

ABSTRACT

Gastrointestinal nematodes (GINs) are ubiquitous in grazing small ruminants and cause significant costs due to production losses. Moreover, anthelmintic resistance (AR) is now widespread throughout Europe and poses a major threat to the sustainability of modern small ruminant livestock farming. Trichostrongylus colubriformis, an intestinal parasite of small ruminants, is the second nematode to Haemonchus contortus in terms of distribution and magnitude in several regions. The control of this helminth is traditionally achieved with the use of anthelmintic drugs, however due to regulations in organic farming and the rise in anthelmintic resistance (AR), alternatives are sought after. The aim of the present study was to evaluate the in vitro anthelmintic effects of olive (Olea Europaea), carob (Ceratonia siliqua), pomegranate juice (Punica granatum), wine (Vitis vibifera), citrus (Citrus Senensis), hazelnut (Corylys avellana) and tomato (Solanum lycopersicum) by-products extracts on T. colubriformis of sheep. Extracts of seven by-products were tested in vitro on two development stages of T. colubriformis (eggs and infective larvae) using the Egg Hatch Assay (EHA) and the Larval Exsheathment Inhibition Assay (LEIA). The egg hatching rate was measured after incubation with each by-product extract (concentrations: 150, 300, 600, 1200 µg/mL) for 48 h at 26 °C. Ensheathed infective larvae were incubated for 3h at 20 °C with each by-product extract (concentrations: 150, 300, 600, 1200 µg/mL). Artificial exsheathment was induced in vitro by adding sodium hypocloride solution (2%w/v) diluted in 1 to 300 in PBS to the larval suspension. The progress of exsheathment over time was measured by repeated observations at 20-min intervals for 60 min (Moreno-Gonzalo et al., 2013. Vet Parasitol, 197:235-43). Among the 7 extracts tested using the two in vitro tests (EHA and LEIA), those with the greatest anthelmintic potential against T. colubriformis were the by-product extracts of wine, pomegranate and hazelnut. In particular, the inhibition in the development of eggs using EHA was 2.3%-48.5% for wine, 6.1%-48.1% for hazelnut and 18.6%-42.4% for pomegranate by-product extracts. The inhibition of exsheathment using LEIA was 87.5%-100% for wine, 54.5%-98.7% for hazelnut and 62.3%-100% for pomegranate by-product extracts. Results showed that by-product extracts of wine, pomegranate and hazelnut exhibits in vitro anthelmintic activity, suggesting that, these by-products can also be an ally for T. colubriformis control in sheep.

Keywords: Gastrointestinal nematodes, *Trichostrongylus colubriformis*, agro-industrial by-products, *in vitro* tests, Egg Hatch Assay, Larval Exsheathment Inhibition Assay

SAŽETAK

Gastrointestinalne nematode (GIN) su sveprisutne u ispaši malih preživara i uzrokuju značajne troškove zbog gubitaka u proizvodnji. Štaviše, otpornost na anthelmintiku (AR) je danas rasprostranjena širom Evrope i predstavlja veliku pretnju održivosti savremenog uzgoja sitnih preživara. Trichostrongilus colubriformis, crevni parazit malih preživara, je druga nematoda posle Haemonchus contortus u smislu distribucije i veličine u nekoliko regiona. Kontrola ovog helminta se tradicionalno postiže upotrebom antihelmintičkih lekova, ali se zbog regulative u organskoj poljoprivredi i porasta anthelmintičke rezistencije (AR), traže alternative. Cilj ove studije bio je da se proceni in vitro anthelmintičko dejstvo masline (Olea Europaea), rogača (Ceratonia silikua), soka od nara (Punica granatum), vina (Vitis vibifera), citrusa (Citrus Senensis), lešnika (Corilis avellana).) i ekstrakti nusproizvoda paradajza (Solanum licopersicum) na T. colubriformis ovaca. Ekstrakti sedam nusproizvoda su testirani in vitro na dve razvojne faze T. colubriformis (jaja i infektivne larve) korišćenjem testa za izležavanje jaja (EHA) i testa inhibicije ekshubicije larve (LEIA). Brzina izleganja jaja je merena nakon inkubacije sa svakim ekstraktom nusproizvoda (koncentracije: 150, 300, 600, 1200 mg/mL) tokom 48 h na 26 °C. Infektivne larve u ovojnici su inkubirane 3 sata na 20 °C sa svakim ekstraktom nusproizvoda (koncentracije: 150, 300, 600, 1200 mg/mL). Veštačko odvajanje je indukovano in vitro dodavanjem rastvora natrijum hipoklorida (2% v/v) razblaženog u 1 do 300 u PBS u suspenziju larve. Napredak eksheathmenta tokom vremena meren je ponovljenim posmatranjima u intervalima od 20 minuta tokom 60 minuta (Moreno-Gonzalo et al., 2013. Vet Parasitol, 197:235-43). Među 7 ekstrakata testiranih korišćenjem dva in vitro testa (EHA i LEIA), oni sa najvećim anthelmintičkim potencijalom protiv T. colubriformis bili su ekstrakti nusproizvoda vina, nara i lešnika. Konkretno, inhibicija u razvoju jaja korišćenjem EHA bila je 2,3%-48,5% za vino, 6,1%-48,1% za lešnik i 18,6%-42,4% za ekstrakte nusproizvoda nara. Inhibicija uklanjanja ovojnice korišćenjem LEIA bila je 87,5% -100% za vino, 54,5% -98,7% za lešnik i 62,3% -100% za ekstrakte nusproizvoda nara. Rezultati su pokazali da ekstrakti nusproizvoda vina, nara i lešnika pokazuju in vitro anthelmintičku aktivnost, što sugeriše da ovi nusproizvodi takođe mogu biti saveznik za kontrolu T. colubriformis kod ovaca.

Ključne reči: gastrointestinalne nematode, *Trichostrongylus colubriformis*, poljoprivredni nusproizvodi, *in vitro* tests, Test leženja jaja, Test inhibicije izbacivanja larvi

INTRODUCTION

Gastrointestinal nematodes (GINs) are increasingly recognized as a significant threat to the future sustainability of keeping livestock for food production and leisure, especially for small ruminant farms (1). At a policy level, livestock significantly contributes to agricultural greenhouse gas (GHG) emissions, which may be exacerbated by GIN infections. GINs also threaten food security and the economic sustainability of livestock farming as they cause significant production losses in ruminants (2). Finally, the impact of subclinical and chronic infections remains an understudied but essential question in livestock helminth research (3).
In particular, Trichostrongylus colubriformis, a nematode of the Trichostrongylidae family which is frequently found in ruminants in many regions worldwide, in severe infections can cause enteritis with atrophy of large intestinal mucosa microvilli, formation of tunnels, and duodenal mucosa epithelium erosion, hyperplasia and crypt hypertrophy, leukocyte infiltration and high intestinal lumen serum protein exudation (4). As a consequence of this infection, the capacity for digestion and absorption of nutrients, including minerals, is compromised, leading to a reduction of skeletal growth, bone density and mineralization, resulting in reduced animal performance (4).
For several decades, the control of these helminth infections has mainly relied on the repeated use of chemical anthelmintics provided by pharmaceutical companies. However, the accelerating development of anthelmintic resistance (AR) in GIN populations and the extent of the phenomenon, including the rise of multi-resistant isolates, have been reported worldwide (5).
Moreover, the development of AR and other chemical drugs appears to be an inevitable process.

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Additionally, anthelmintics can be included among the "emerging pollutants" that have significantly risen in the last decades (6). Their ecotoxicological effects have raised concern in relation to environmental invertebrates, which can be irremediably damaged (7). At the same time, anthelmintics can indirectly affect human and animal health by reaching ground and surface waters and land resources or by accumulation in the food chain (6).
For these reasons, it is essential to develop innovative strategies in modern sheep and goat farming systems that are based on: i) the rational use of the drug as "targeted selective treatment (TST)" for animals clinically diagnosed with GINs to leave some parasite populations unexposed to anthelmintics (refugia), ii) the use resistant/resilient breeds to GIN infections, iii) the use of plants with anthelmintic properties and iv) the integration of sensitive diagnostic techniques able to early detect the onset of AR phenomenon.
Currently the most eco-sustainable solution is the use of plants with anthelmintic activity to GIN control. The aim of the present study was to evaluate the in vitro anthelmintic effects of olive (Olea Europaea), carob (Ceratonia siliqua), pomegranate (Punica granatum), wine (Vitis vibifera), citrus (Citrus Senensis), hazelnut (Corylys avellana) and tomato (Solanum lycopersicum) by-products extracts on T. colubriformis of sheep.

MATERIAL AND METHODS

The by-products used in this study were supplied by agro-industries present in southern Italy. Particularly olive, pomegranate, grape pomace, hazelnut and tomato by-products derive from agro-industries located in Campania region, instead carob and citrus by-products derive from agro-industries located in Sicily region.

Preparation of extracts

The method of extraction has been described previously (8). Briefly, 500 g of the whole waste were extracted with 2X3L of 70 :30 acetone : water (v/v) containing ascorbic acid (1 g/l) for 24 h. The acetone was removed under low pressure at a temperature < 35° C and the aqueous solution was washed 4 times with 500 ml of methylene chloride to remove chlorophyll and lipids. The solution was then concentrated under low pressure at 35 ° C. Finally, the extracts were frozen and lyophilized for 24 h to obtain a dry ground sample which was used in the *in vitro* biological assay.

In vitro assays

Third-stage larvae (L3) and eggs were obtained from feces of monospecifically infected sheep donor, with susceptible strains of *T. colubriformis*. L3 larvae were maintained in culture flasks for 2 months, at 4°C, before use in the Larval Exsheathment Inhibition Assay (LEIA), while eggs were collected on the day of the Egg Hatch Assay (EHA), and used up to 3 h after collection (9, 10).

The extracts were evaluated for the AH activity by using the *in vitro* Larval Exsheathment Inhibition Assay (LEIA) (11) and the EHA was conducted following the procedure suggested by (9, 10). Each assay was applied successively on a species of intestinal nematode (*T. colubriformis*). The two assays were performed with the same range of concentrations of 1200, 600, 300, and 150 µg/ml using Phosphate Buffered Saline (PBS) (0.1 M phosphate, 0.05 M NaCl, pH 7.2) as the solvent. For each concentration, including the negative and positive controls, assays were performed in four replicates.

For the EHA, twenty-four well culture plates were used for the *in vitro* anthelmintic test of the plant extracts against the eggs of *T. colubriformis*. The anthelmintic test against the eggs was evaluated separately for each plant extract.

In a 24 well culture plate, there were prepared 4 replicates of the same plant extract in the following concentrations: PBS control (without any plant extract), 150µg/ml, 300µg/ml, 600µg/ml, 1200µg/ml and TBZ (which is the golden standard for the egg hatch of the nematodes). The eggs and the larvae were counted for the four replicates and an average value was estimated, converted into percentage of inhibition of egg hatch.

For the LEIA, the third-stage larvae of *T. colubriformis* were produced from experimentally infected donor sheep. The same batch of 2 to 3-month-old larvae was used in the assays with different extracts. Firstly 1000 L3/ml solution were diluted at concentrations of 1200µg/ml, 600µg/ml, 300µg/ml and 150µg/ml plant extracts with 4 replicates in each concentration. Negative controls (L3 in PBS) were used as control of the assay. The ensheathed L3-larva were incubated for 3h at 20°C

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with the plant extracts before being resuspended at the concentrations of 1200µg/ml, 600µg/ml, 300µg/ml and 150µg/ml in phosphate buffer solution (PBS). After incubation, the larvae were washed and centrifugated, three times in PBS. After the incubation with the plant extracts, the larvae were submitted to the artificial process of exsheathment by contact with a sodium hypocloride solution (2%w/v) diluted in 1 to 400 in PBS.

The larvae kinetics and morphology were evaluated with microscopic observation and identification of exsheathed larvae at a magnification of x 200 by regular examination at 0, 20, 40 and 60 minutes of contact with the solution for exsheathment. Four replicates were run for each plant extract at the concentrations 1200µg/ml, 600µg/ml, 300µg/ml and 150µg/ml as long as the negative control to examine the changes in proportion of exsheathed larvae with time.

RESULTS

The highest ovicidal effect was obtained with grape and olive by-product extracts which had a same percentage of inhibition of egg hatch of 48.5%. The best results were also obtained with hazelnut (48.1% higher inhibition), tomato (43.2% higher inhibition) and pomegranate (42.4% higher inhibition) by-product extracts. The worst results were obtained with carob and citrus by-product extracts (< 16.3%).

Concerning larvicidal activity, the results obtained showed that the greatest anthelmintic potential against *T. colubriformis* were the by-product extracts of wine, pomegranate and hazelnut. Specifically, with pomegranate extract when the larvae were exposed to extract concentration of 1200 µg/ml and 600 µg/ml, the inhibition of exsheathment of L3 was 100%, furthermore, even at a concentration of 300 µg/ml only 15.8% of the larvae lost their sheath.

With the grape extract the inhibition of exsheathment using LEIA was 100% when larvae were exposed to extract concentration of 1200 µg/ml and 600 µg/ml, after 60 minutes, 89.6% at a concentration of 300 µg/ml and 87.5% at 150 µg/ml.

Hazelnut by-product extracts also gave excellent results because when the larvae were exposed to a

extract concentration of 1200 µg/ml, 600 µg/ml and 300 µg/ml, the lowest inhibition value was 98.2%.

Good results were also obtained with olive (81.7% higher inhibition), carob (90.0% higher inhibition) and tomato (100% higher inhibition) by-products.

The least encouraging results in this study were obtained with by-products deriving from citrus.

DISCUSSION

The use of extracts from plants has been extensively studied as an alternative to anthelmintics in the treatment of gastrointestinal parasites in sheep (12), as they may be sources of phytoconstituents, which display various biological proper- ties (13).

In the context of growing resistance to antihelmintic drugs, the potential of agro-industrial by-products against pathogens represents an alternative more sustainable than the use of synthetic drugs. Several *in vitro* studies have demonstrated the potential benefits arising from the inclusion of bioactive products in feed for ruminants and for the future use as nutraceuticals. Many of these studies have been performed on *T. colubriformis* (12).

The aim of the present study was to evaluate the *in vitro* efficacy of different agro-industrial by-products containing plant specialised metabolites (PSM), studying their potential bioactive.

Even if in a different way, all the products used have proven to have a certain anthelmintic activity on eggs and on L3 larvae of *T. colubriformis*, which was significant in some of the extracts (pomegranate extract, grape pomace extract, hazelnut extract), medium in others (olive extract, carob extract, tomato extract) and only in one extract was it shown to be low (citrus extract).

The different efficacy of the substances against GIN could be associated with qualitative and quantitative differences in phytochemical compounds. In particular, during the tests carried out, it was noted that the effectiveness of the products tested was dose-dependent and time-dependent.

Studies on the *in vitro* anthelmintic efficacy of pomegranate by-products (14), tested a watery macerate obtained from the fruits and their peels, obtaining a significant ovicidal activity (assessed with EHT) against GIN of sheep. These results are in agreement with the *in vitro* and *in vivo* studies conducted by Hassan et al. (2020) (15), who confirmed the effectiveness of *P. granatum* extract against *Haemonchus contortus* in goats and *Haemonchus* spp. and *Cooperia* spp. in cattle. These studies confirm the results obtained in this study.

An *in vitro* study by Soares et al. (2018) (16) evaluated ovicidal and larvicidal activity of a hydroalcoholic extract of grape, against gastrointestinal nematodes, on naturally infected sheep. The pomace extract showed a high ovicidal and larvicidal activity *in vitro* with LD50 values of 0.30 mg/ml for inhibition of egg hatching, 1.01 mg/ml for inhibition of development larval migration and 100% efficacy in all larval migration inhibition tests the concentrations evaluated. The present study also demonstrated that these by-products are one potential source of bioactive compounds with significant larvicidal properties *in vitro*.

As reported by Hoste et al. (2015) (17), has been compared in some studies the *in vitro* effectiveness of sainfoin and hazelnut extracts, thus allowing a comparison of IC50 values (18, 19). In the overall, lower IC50 values were obtained for hazelnuts, suggesting a higher anthelmintic activity than sainfoin, which is used as a reference among legume plants containing tannins. In another study the anthelmintic properties were confirmed *in vivo* (12). When goats experimentally infected with *H. contortus* and *T. colubriformis*, were fed hazelnut peels, was observed a significant reduction (>50%) in fecal nematode egg counts (FEC), related to a decrease in the fecundity of *Haemonchus* females (20). In a second phase, decreases were recorded in L3 larvae by 48% for *H. contortus* and 42% for *T. colubriformis*. In accordance with these studies, the results obtained in laboratory with the LEIA and EHA, have shown a high anthelmintic potential.

CONCLUSIONS

The present study aimed to investigate the antihelmintic potential of various agro-industrial by-products, in particular from the olive, wine, canning and fruit (citrus, carob, hazelnut and pomegranate). Despite being necessary to continue with further studies to confirm the results reached, it can be stated in the first instance that the extracts deriving from the by-products of winemaking, from the processing of hazelnuts and pomegranate have revealed themselves very effective against eggs and L3 larvae of *T. colubriformis*. The by-products follow of the processing of olives, tomatoes and carobs, which have shown an effectiveness that should not be underestimated, even if, to a lesser extent, compared to the previous ones. It follows that these substances could represent in future, a really promising alternative for them to be used as raw material to create new marketable products with activities anthelmintic, in order to achieve sustainable infection control parasitic.

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REFERENCES

1. *Rose Vineer H., Verschave S.H., Claerebout E., Vercruysse J., Shaw D.J., Charlier J., Morgan E.R. GLOWORM-PARA: a flexible framework to simulate the population dynamics of the parasitic phase of gastrointestinal nematodes infecting grazing livestock. Int J Parasitol. 2020, 50(2):133-144.*
2. *Charlier J., Bartley D.J., Sotiraki S., Martinez-Valladares M., Claerebout E., von Samson-Himmelstjerna G., Thamsborg S.M., Hoste H., Morgan E.R., Rinaldi L. Anthelmintic resistance in ruminants: challenges and solutions. Adv Parasitol. 2022, 115:171-227.*
3. *Morgan E.R., Lanusse C., Rinaldi L., Charlier J., Vercruysse J. Confounding factors affecting faecal egg count reduction as a measure of anthelmintic efficacy. Parasite 2022, 29:20.*
4. *Dias E Silva T.P., Ventoso Bompadre T.F., Danasekaran D.K., Sakita G.Z., Abdalla Filho A.L., Jimenez C.R., de Campos Fonseca Pinto A.C.B., do Amarante A.F.T., McManus C., Louvandini H. Trichostrongylus colubriformis infection: Impact on digesta passage rate and lamb performance. Vet Parasitol. 2019, 272:17-22.*
5. *Rose Vineer H., Morgan E.R., Hertzberg H., Bartley D.J., Bosco A., Charlier J., Chartier C., Claerebout E., de Waal T., Hendrickx G., Hinney B., Höglund J., Ježek J., Kašný M., Keane O.M., Martínez-Valladares M., Mateus T.L., McIntyre J., Mickiewicz M., Munoz A.M., Phythian C.J., Ploeger H.W., Rataj A.V., Skuce P.J., Simin S.,*

ISBN 978-86-7520-611-8

Sotiraki S., Spinu M., Stuen S., Thamsborg S.M., Vadlejch J., Varady M., von Samson-Himmelstjerna G., Rinaldi L. Increasing importance of anthelmintic resistance in European livestock: creation and meta-analysis of an open database. Parasite 2020, 27:69.
6. *Horvat A.J.M., Babić S., Pavlović D.M., Ašperger D., Pelko S., Kaštelan-Macan M., Petrović M., Mance A.D. Analysis, occurrence and fate of anthelmintics and their transformation products in the environment. Trends in Analytical Chemistry 2012, 31:61-84.*
7. *Beynon S.A. Potential environmental consequences of administration of anthelmintics to sheep. Vet Parasitol. 2012, 189(1):113-24.*
8. *Barrau E., Fabre N., Fouraste I., Hoste H. Effect of bioactive compounds fromsainfoin (Onobrychis viciifolia) on the in vitro larval migration of Haemonchus contortus: Role of tannins and flavonolglycosides. Parasitology 2005, 131:531-538.*
9. *Coles G.C., Bauer C., Borgsteede F.H., Geerts S., Klei T.R., Taylor M.A., Waller P.J.World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol.1992, 44, 35-44.*
10. *Jackson F., Hoste H. In vitro methods for the primary screening of plant products for direct activity against ruminant gastrointestinal nematodes. In: In Vitro Screening of Plant Resources for Extra-nutritional Attributes in Ruminants: Nuclear and Related Methodologies, 2010.*
11. *Bahuaud D., Martinez-Ortiz De Montellano C., Chauveau S., Prevot F., Torres Acosta F., Fouraste I., Hoste H. Effects of four tanniferous plant extracts on the in vitro exsheathment of third-stage larvae of parasitic nematodes. Parasitology 2006, 132(Pt 4), 545-54.*
12. *Hoste H., Meza-OCampos G., Marchand S., Sotiraki S., Sarasti K., M. Blomstrand M., R. Williams A., M. Thamsborg S., Athanasiadou S., L. Enemark H., Torres Acosta J.F., Mancilla-Montelongo G., Castro CS., M. Costa-Junior, L. Louvandini H., Sousa D.M., Salminen J-P., Karonen M., Engstrom M., Charlier J., Niderkorn V., R. Morgan E. Use of agro-industrial by-products containing tannins for the integrated control of gastrointestinal nematodes in ruminants. Parasite 2022, 9-11.*
13. *Djilas S., Jasna C.B., Ćetković G. By-products of fruits processing as a source of phytochemicals. Chem Ind Chem Eng Q 2009, 15,191–202.*
14. *Castagna F., Britti D., Oliverio M., Bosco A., Bonacci S., Iriti G., Ragusa M., Musolino V., Rinaldi L., Palma E., Musella V. In Vitro Anthelminthic Efficacy of Aqueous Pomegranate (Punica granatum L.) Extracts against Gastrointestinal Nematodes of Sheep. Pathogens. 2020, 9, 1063.*
15. *Hassan N.M.F., Sedky D., Abd El-Aziz T.H., Shalaby H.A., Abou-Zeina H.A.A. Anthelmintic potency and curative effect of pomegranate peels ethanolic extract against H. contortus infection in goats. Int J Vet. Sci. 2020, 9(2), 210-216.*
16. *Soares S.C.S., de Lima G.C., Laurentiz A.C., Féboli A., dos Anjos L.A., de Paula Carlis M.S., da Silva Filardi R., da Silva de Laurentiz R. In vitro anthelmintic activity of grape pomace extract against gastrointestinal nematodes of naturally infected sheep. International Journal of Veterinary Science and Medicine 2018, 6, 243–247.*
17. *Hoste H., Torres-Acosta J.F.J., Sandoval-Castro C.A., Mueller-Harvey I., Sotiraki S., Louvandini H., Thamsborg S.M., Terrill T.H. Tannin containing legumes as a model for nutraceuticals against digestive parasites in livestock. Veterinary Parasitology 2015, 212, 5–17.*
18. *Boudot C. Étude in vitro de l’activité anthelminthique de coproduits agro industriels riches en tanins et flavonoïdes. Report for the grade of Pharmacian Faculty of Pharmacy Univ Paul Sabatier Toulouse, 2014.*
19. *Quijada J., Fryganas C., Ropiak H.M., Ramsay A., Mueller-Harvey I., Hoste H. Anthelmintic activities against Haemonchus contortus or Trichostrongylus colubriformis from small ruminants are influenced by different structural features of condensed tannins. Journal of Agricultural Food and Chemistry 2015, 63, 6346–6354.*
20. *Desrues O., Vargas-Magana J., Girard M., Manolaraki F., Pardo E., Mathieu C., Vilarem G., Torres Acosta J.F.J., Sandoval Castro C.A., Jean H., Hoste H. Can hazel-nut peels be used to control gastrointestinal nematodes in goats? International Goat Association (IGA) Conference. 4th CAPARA Conference, Las Canarias, Book of abstract, p. 44, 2012.*

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Article
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KRVNA SLIKA KOD KRAVA U PERIPARTALNOM PERIODU I ODNOS PREMA POKAZATELJIMA METABOLIČKOG STRESA

COMPLETE BLOOD COUNT IN COWS IN THE PERIPARTAL PERIOD AND THE RELATIONSHIP TO THE INDICATORS OF METABOLIC STRESS

Marko Cincović[a], Jože Starič[b], Jožica Ježek[b], Mira Majkić[a], Sandra Nikolić[a], Bojan Blond[c], Nemanja Obradović[d], Dražen Kovačević[e]

[a]Departman za veterinarsku medicinu, Poljoprivredni fakultet, Univerzitet u Novom Sadu, Srbija
[b]Veterinarski fakultet, Univerzitet u Ljubljani, Slovenija
[c]ALDAHRA Beograd, Srbija
[d]Pasterov zavod Novi Sad, Srbija
[e]Veterinarska inspekcija Opštine Teslić, R.Srpska, BiH
*Autor za korespondenciju: mcincovic@gmail.com

M.C. https://orcid.org/0000-0002-5683-8983, J.S. https://orcid.org/0000-0002-5156-5277, J.J. https://orcid.org/0000-0002-3753-5944, M.M. https://orcid.org/0009-0007-0110-7327, S.N. https://orcid.org/0000-0001-7337-9937,

SAŽETAK

Laktacija i metaboličko prestrojavanje značajno utiču na hematološke parametre. Crvena krvna loza krava u ranoj laktaciji odlikuje se hipohromnim promenama u eritrocitima i smanjenim brojem eritrocita. Snižena koncentracija hemoglobina se dovodi u vezu sa proizvodnjom mleka, obzirom da je kod junica (koje prirodno nisu u laktaciji) nađena veća koncentracija hemoglobina. Broj neutrofila bio je najveći u prvoj nedelji posle partusa, dok je broj limfocita bio najniži u istom periodu, tako da je indeks neutrofili:limfociti bio najviši upravo u ovom periodu. Broj eozinofila je blago rastao tokom oglednog perioda, dok je broj monocita bio najveći u prvoj nedelji da bi potom opadao. Ovakve promene u diferencijalnoj beloj lozi nastaju kao posledica delovanja akutnog stresa i naglog skoka koncentracije kortizola koji je pokazao svoj uticaj na imune ćelije. Kod bele loze NEFA kao indikator metaboličkog stresa dovodi do pada ukupnog broja leukocita, a krave klasifikovane prema kriterijumu NEFA i kriterijumu kortizola pokazuju povećan procenat neutrofila, smanjen procenat limfocita, povećan N:L odnos, smanjen procenat eozinofila i povećan procenat monocita. Uticaj kortizola na parametre krvne slike samo u prvoj nedelji postoji jer koncentracija kortizola naglo poraste, a potom i naglo opadne u prvih nekoliko dana po teljenju, dok se koncentracija NEFA penje lagano i često je perzistentna u prvim nedeljama laktacije, što ukazuje na negativni energetski bilans. U ispitivanju uticaja indikatora metaboličkog stresa na vrednost parametara krvne slike nađeno je da klasifikacija krava prema vrednostima NEFA i kortizola daje signifikantne razlike u crvenoj i beloj krvnoj lozi, što potvrđuje značaj peripartalnog metaboličkog stresa koji je izazvan partusom i laktacijom u odgovoru krvne slike kod krava.

Ključne reči: krave, krvna slika, metabolički stres, laktacija.

ABSTRACT

Lactation and metabolic rearrangement significantly influence hematological parameters. The red bloodline of early lactation cows is characterized by hypochromic changes in erythrocytes and a reduced number of erythrocytes. The reduced concentration of hemoglobin is linked to milk production, since a higher hemoglobin concentration was found in heifers (which are not naturally lactating). The number of neutrophils was the highest in the first week after parturition, while the number of lymphocytes was the lowest in the same period, so the neutrophil:lymphocyte index was the highest in this period. The number of eosinophils increased slightly during the experimental period, while the number of monocytes was the

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highest in the first week and then decreased. Such changes in the differential white line occur as a consequence of the action of acute stress and a sudden jump in the concentration of cortisol, which has shown its effect on immune cells. In white cattle, NEFA as an indicator of metabolic stress leads to a drop in the total number of leukocytes, and cows classified according to the NEFA and cortisol criteria show an increased percentage of neutrophils, a decreased percentage of lymphocytes, an increased N:L ratio, a decreased percentage of eosinophils and an increased percentage of monocytes. The influence of cortisol on the parameters of the blood count only exists in the first week because the concentration of cortisol rises sharply and then sharply decreases in the first few days after calving, while the concentration of NEFA rises slightly and is often persistent in the first weeks of lactation, which indicates a negative energy balance . In the study of the influence of metabolic stress indicators on the value of the parameters of the blood count, it was found that the classification of cows according to the values of NEFA and cortisol gives significant differences in the red and white bloodlines, which confirms the importance of peripartum metabolic stress caused by parturition and lactation in the response of the blood count in cows.

Key words: cows, complete blood count, metabolic stress, lactation.

KRVNA SLIKA I NJENA INTERPERTACIJA KOD KRAVA

Krvna slika kod mlečnih krava, kao i kod drugih životinjskih vrsta ima velikog značaja u proceni zdravlja. Analiza standardne krvne slike crvene loze podrazumeva: 1. ispitivanje hematološkog statusa (određivanje hematokrita, broja eritrocita, koncentracije hemoglobina, eritrocitnih indeksa); 2. ispitivanje biohemijskog statusa (serumsko gvožđe, feritin, kapacitet vezivanja gvožđa i slobodni transferin); 3. ispitivanje markera hemolize (nekonjugovani bilirubin, hemoglobin u urinu, haptoglobin, hemosiderin u urinu); 4. ispitivanje imunoloških markera (Coombsov test, eritrocitna antitela i sl.) (1).

U brojnim anemijama se javlja smanjena koncentracija hemoglobina. Za detaljniju dijagnostiku bitno je odrediti eritrocitne indekse: MCV (eng., *mean cell volume*, srednja vrednost zapremine eritrocita), MCH (eng., *mean cell-corpuscular hemoglobine*, srednja vrednost količine hemoglobina u ćeliji), MCHC (eng., *mean cell-corpuscular hemoglobine concentration*, srednja vrednost koncentracije hemoglobina u eritrocitima). Za potpunu analizu crvene krvne loze potrebno je ispitati i postojanje anizocitoze i poikilocitoze analizom mikroskopskog razmaza (2,3). Kod mlečnih krava brojni faktori imaju uticaj na vrednosti crvene krvne loze. Tako mlečne krave imaju niže vrednosti parametara eritrocitne loze u odnosu na tovne rase, dok u okviru mlečnih rasa najnižu vrednost hemoglobina ima crno-beli Holštajn. Mlečnost ima značajnu ulogu, tako da sa rastom mlečnosti opada vrednost hemoglobina i hematokrita. U peripartalnom periodu, posebno na sam dan porođaja i u prvim danima laktacije krave imaju manji broj eritrocita i nižu kocnentraciju hemoglobina (4-7). Nastanak anemije i snižena koncentracija hemoglobina uz metaboličko opterećenje i povišenu koncentraciju NEFA mogu predisponirati krave ka nastanku peripartalne inflamacije uterusa (8). Ketoza kod krava u peripartalnom periodu ima uticaja na eritrocitnu lozu koja može pokazati znake anemije (9). Intenzitet lipolize i ketogeneze u peripartalnom periodu je u negativnoj korelaciji sa vrednostima crvene krvne loze (10).

Povećan ukupan broj leukocita (leukocitoza) nastaje usled: inflamacije, nekroze tkiva, delovanja kortikosteroida i epinefrina, akutne limfoblasne leukemije, hronične limfocitne leukemije, akutne ili hronične mijeloidne leukemije, limfoma i deficijencije adhezionih molekula leukocita. Smanjenje ukupnog broja leukocita (leukopenija) se javlja prilikom: jake infekcije, endotoksičnih stanja, aplastične anemije, aleukemičnih akutnih leukemija, infektivnih bolesti, mijelodisplastični sindrom i dr. Pored ukupnog broja leukocita značajno je odrediti i diferencijalnu krvnu sliku sa brojem bazofila, neutrofila, eozinofila, monocita i limfocita. Oni se mogu odrediti pomoću hematološkog brojača. Povećan broj bazofila (bazofilija) ukazuje na hipersenzitivne reakcije, tumor mast ćelija, parazitizam, bazofilnu leukemiju i mijeloproliferativne bolesti. Bazopenija se vrlo teško dijagnostikuje, jer vrednost od nula bazofila kod većine životinjskih vrsta i ljudi ulazi u referentne vrednosti. Eozinofilija se javlja u hipersenzitivnim

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reakcijama, parazitozama, limfomima, hipoadrenokorticizmu, eozinofilnoj leukemiji i dr. Eozinopenija je teška za dijagnostiku, jer nula ulazi u referentnu vrednost, ali hronična eozinopenija ukazuje na insuficijenciju koštane srži. Limfocitoza ukazuje na hroničnu antigensku stimulaciju, nelimfoidne neoplazme, imunski posredovane bolesti, limfocitne i limfoblasne leukemije. Limfopenija nastaje kao posledica delovanja glukokortikoida, virusnih infekcija, imunosupresije, kod enteropatija sa gubitkom proteina, imunodeficijentnih stanja i sl. Monocitoza prati septikemična stanja, hemolize, odgovor na glukokortikoide, nehematopoezne neoplazme i oštećenje kostne srži, dok se monocitopenija teško dijagnostikuje. Neutrofilija se javlja kod inflamacija, kao odgovor na glukokortikoide i epinefrin, zatim kod hronične mijeloidne leukemije i deficijencije adhezionih molekula leukocita. Neutropenija se dešava kod endotoksemija, aplastične anemije, aleukemičnim leukemija, virusnih infekcija, imunoposredovanih stanja i dr. Pored određivanja broja leukocita i diferencijalne krvne slike značajno je odrediti i njihovu starost. Ona se određuje na osnovu prisustva blasta i stepena segmentiranosti njihovog jedra. Ako se u perifernoj krvi javljaju mlađi oblici razvoja granulocitne loze, sa velikim brojom štapastih granulocita i metamijelocita, onda se takva pojava zove skretanje u levo. Skretanje u levo uz leukocitozu govori u prilog aktivnoj produkciji ćelija bele loze, ali je skretanje u levo uz leukopeniju prognostički vrlo loš znak kod mnogih bolesti (2,3).

Kod mlečnih krava laktacija ne utiče značajno na parametre bele krvne loze, ali endokrinološke promene, peripartalni stres i razvoj infekcija značajno menjaju krvu sliku krava u peripartalnom periodu. Od posebnog interesa je određivanje ukupnog broja leukocita, broja neutrofila, limfocita i odnosa broja neutrofila prema broju limfocitia (N:L odnos). U peripartalnom priodu dolazi do opadanja ukupnog broja leukocita (4-7). Pad broja leukocita slabi odbrambene sposobnosti organizma, a krave su posebno predisponirane mastitisu ako imaju nisku vrednost eozinofila, odnosno metritisu ako postoji niska vrednost monocita (8).

Zapaljenske reakcije i diferencijalna bela loza kod krava moraju se drugačije posmatrati, jer postoje specifičnosti krvne slike i to: visok procenat limfocita (60-80%) i slaba ili nikakava rezerva mladih granulocita u kostnoj srži. Tokom zapaljenske reakcije kod goveda dolazi do pada broja neutrofilnih granulocita uz pad ukupnog broja leukocita, pa su neutropenija i leukopenija prvi znaci u akutnoj upali. Tek nakon nekoliko dana ubrzava se leukopoeza i mladi neutrofilni granulociti izlaze u cirkulaciju, ali je klinički često neprimećen zbog brzog trošenja. U akutnim upalama gnojnog tipa postoji leukopenija, limfopenija i neutropenija sa skretanjem u levo. Ako životinja preživi akutnu fazu dešavaju se kompenzacije u beloj lozi, tako da raste broj neutrofilnih granulocita a broj limfocita opada, pa njihov odnos postaje inverzan (11). Ovdse su opisane promene u klasičnoj zapaljenskoj reakciji. Peripartalni period pokazuje određene specifičnosti kada je odnos neutrofila i limfocita u pitanju.

Odnos neutrofila i limfocita (N:L odnos) je značajan pokazatelj zdravlja i stresne opterećenosti životinja (12). Ovaj odnos kod odraslih goveda iznosi približno 0,5:1. U periodu oko teljenja kod krava postoji tipičan stresni leukogram, koji se odlikuje neutrofilijom, limfopenijom, eozinopenijom i varijabilnom monocitozom. Akutni stres i hiperkortizolemija dovode do porasta koncentracije neutrofila povlačenjem ovih ćelija u centralni pul krvotoka, dok limfociti migriraju ka periferiji, tako da dolazi do porasta odnosa neutrofila i limfocita (N:L odnos često preko 1) (13). Posle adaptacije na stresne uslove N:L odnos se vraća na fiziološki nivo u periodu 2-7 dana (14). Manja vrednost N:L odnosa neđena je na farmama sa dobrom negom i dobrim medicinskim tretmanom krava (15). Novija istraživanja pokazuju da N/L odnos može biti veći od 1 kod potpuno zdravih krava, pa se upotreba ovog indikatora mora vršiti uz sagledavanje kompletnog zdravlja i metabolizma krava (16). Krave kod kojih je 7-15 dana posle teljenja N:L odnos preko fizioloških vrednosti nalaze se u fazi prolongiranog stresa. Prolongirani stres može biti uzrokovan prolongiranim metaboličkim stresom sa povišenim vrednostima NEFA i BHB i sniženom vrednošću glikemije, koji se javlja kod određenog broja krava posle teljenja. Koncentracija ovih metabolita značajno korelira sa N:L odnosom (17). Povišene vrednosti stresnih hormona dovode do porasta neutrofila i ulaska u krvotok iz marginalnog pula. U jednom ogledu ispitan je uticaj aplikacije deksametazona na funkciju limfocita u drugoj nedelji posle partusa. Aplikacija deksametazona dovela je do značajnog porasta N:L odnosa, koji je predhodno bio u okviru fizioloških granica, na račun porasta broja neutrofila. Dakle, veštačkim podražavanjem akutne stresne reakcije dolazi do

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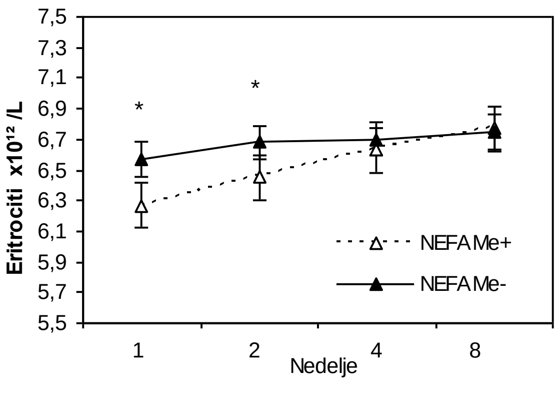
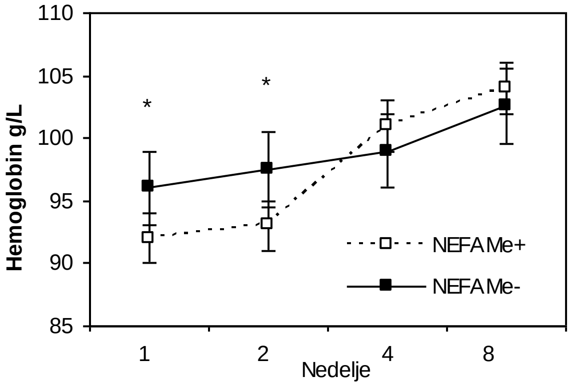
značajnog porasta broja neutrofila u nedeljama posle partusa (18). Vrednost odnosa N:L preko 1 ukazuje da su krave opterećene inflamacijom ili drugim stresorima (19).

METABOLIČKI STRES I KRVNA SLIKA KRAVA

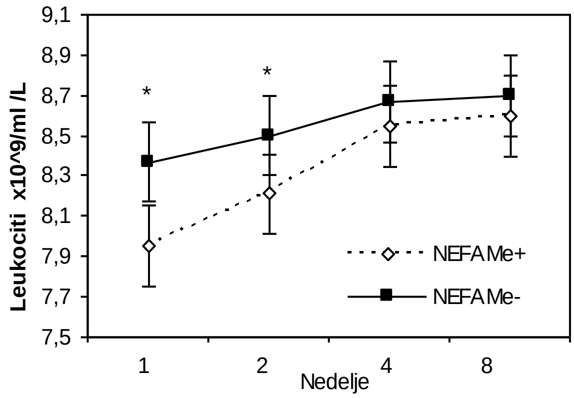
U ispitivanju uticaja indikatora metaboličkog stresa na vrednost parametara krvne slike nađeno je da klasifikacija krava prema vrednostima NEFA i kortizola daje signifikantne razlike u crvenoj i beloj krvnoj lozi. Krave koje pripadaju NEFA^{Me+} grupi pokazuju manji broj erirtocita, nižu koncentraciju hemoglobina, manji broj leukocita, veći broj neutrofila i veći odnos N:L u provoj i drugoj nedelji posle partusa u odnosu na krave NEFA^{Me-} grupe. Krave koje pripadaju kortizol^{Me+} grupi pokazuju tipičan stresni leukogram u prvoj nedelji posle teljenja, pa one imaju veći broj neutrofila, manji broj limfocita, manji broj eozinofila i veći broj monocita, kao i veći N:L odnos. Klasifikacija prema kombinovanom uticaju anaboličkih i kataboličkih indikatora stresa nije dala signifikantne razlike. Pored signifikantnog uticaja NEFA i kortizola na krvnu sliku i diferencijalnu belu lozu, isti uticaj postoji i kada je u pitanju vijabilnost leukocita. Kod krava opterećenih metaboličkim stresom (NEFA^{Me+} i kortizol^{Me+}) postoji niža vijabilnost leukocita u prvoj i drugoj nedelji posle teljenja u odnosu na krave iz grupe NEFA^{Me-} i kortizol^{Me-}. Svi rezultati su prikazani na Grafikonima 1-9 u nastavku.



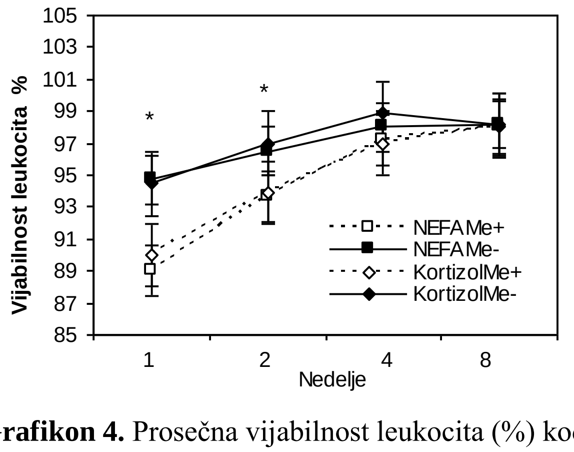
Grafikon 1. Prosečan broj eritrocita kod krava klasifikovanih prema NEFA kriterijumu



Grafikon 2. Prosečna koncentracija hemoglobina kod krava klasifikovanih prema NEFA kriterijumu



Grafikon 3. Prosečan broj leukocita kod krava klasifikovanih prema NEFA kriterijumu



Grafikon 4. Prosečna vijabilnost leukocita (%) kod krava klasifikovanih prema kriterijumu NEFA i kortizol

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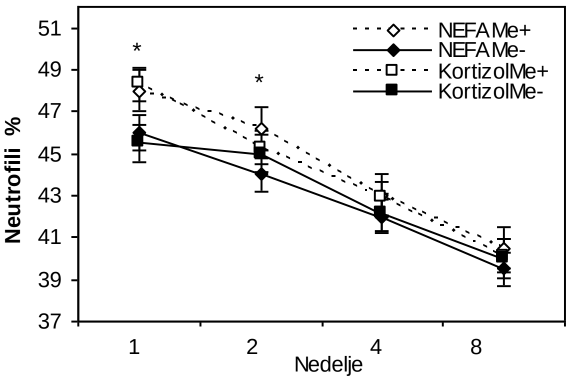

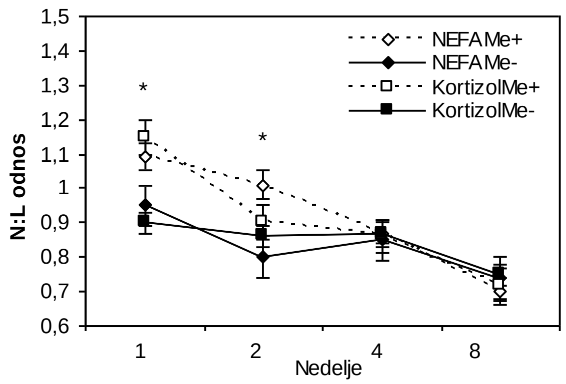

Grafikon 5 i 6. Prosečan % neutrofila i vrednost odnosa N:L kod krava klasifikovanih prema kriterijumima NEFA i kortizol

Crvena krvna loza krava u ranoj laktaciji odlikuje se hipohromnim promenama u eritrocitima i smanjenim brojem eritrocita. Snižena koncentracija hemoglobina se dovodi u vezu sa proizvodnjom mleka, obzirom da je kod junica (koje prirodno nisu u laktaciji) nađena veća koncentracija hemoglobina. Broj neutrofila bio je najveći u prvoj nedelji posle partusa, dok je broj limfocita bio najniži u istom periodu, tako da je indeks neutrofili:limfociti bio najviši upravo u ovom periodu. Broj eozinofila je blago rastao tokom oglednog perioda, dok je broj monocita bio najveći u prvoj nedelji da bi potom opadao. Ovakve promene u diferencijalnoj beloj lozi nastaju kao posledica delovanja akutnog stresa i naglog skoka koncentracije kortizola koji je pokazao svoj uticaj na imune ćelije. Drugi bitan faktor je u vezi sa velikim izmenama u uterusu i vimenu koje se dešavaju posle partusa i u ranoj laktaciji, gde imunološke ćelije odlaze kako bi zaštitile ove vrlo osetljive organe. Rezultati krvne slike su u skladu sa ranije dobijenim rezultatima (11,16). Rezultati istraživanja pokazuju da katabolički indikatori stresa kao što su kortizola i NEFA utiču na hematološke vrednosti i vijabilnost leukocita. Krave sa višim vrednostima NEFA imaju manji broj eritrocita i nižu koncentraciju hemoglobina u prve dve nedelje posle teljenja. Kod krava u ranoj laktaciji kortizol ima značajnu ulogu u započinjanju laktacije, a viša koncentracije NEFA je u vezi sa smanjenim unosom hrane. Mlečnost krava je u inverznom odnosu prema vrednostima hemoglobina i hematokrita. U peripartalnom periodu, posebno na sam dan porođaja i u prvim danima laktacije krave imaju manji broj eritrocita i nižu kocnentraciju hemoglobina (4-7). Uhranjenost krava (telesna kondicija) u peripartalnom periodu pozitivno korelira sa vrednostima hemoglobina, MVC, MCH i hematokrita (20). Uticaj lučenja mleka i smanjen unos hrane u ranoj laktaciji mogu biti u vezi sa smanjenim brojem eritrocita i koncentracijom hemoglobina.

Kod bele loze NEFA kao indikator metaboličkog stresa dovodi do pada ukupnog broja leukocita, a krave klasifikovane prema kriterijumu NEFA i kriterijumu kortizola pokazuju povećan procenat neutrofila, smanjen procenat limfocita, povećan N:L odnos, smanjen procenat eozinofila i povećan procenat monocita. Uticaj kortizola na parametre krvne slike samo u prvoj nedelji nastaje jer koncentracija kortizola naglo poraste, a potom i naglo opadne u prvih nekoliko dana po teljenju, dok se koncentracija NEFA penje lagano i često je perzistentna u prvim nedeljama laktacije, što ukazuje na negativni energetski bilans. Glukokortikoidi dovode do tipičnog stresnog leukograma tako što izazivaju umanjenu blastogenezu leukocita, povećan broj neutrofila preko L-selektina i β2-integrina, što smanjuje mogućnost neutrofila da napuste cirkulaciju ili dovode do povećanog slanja neutrofila iz kostne srži u krvotok (18). Negativni energetski bilans menja genetičke karakteristike imunoloških ćelija dovodeći do brojnih izmena u ekspresiji gena koji umanjuju njihov funkcionalni status (21).

Kod krava sa metritisom neutrofili imaju manju koncentraciju glikogena, što se takođe može povezati sa negativnim energetskim bilansom i metaboličkim stresom čiji su indikatori u našem modelu NEFA i kortizol (22). Metabolički stes u ranoj laktaciji se karakteriše smanjenom koncentracijom insulina i IGF-I. Izlaganje neutrofila ovim hormonima povećava njihovu kompetencu i učinak (23). Insulin utiče na transportere za glukozu u leukocitima, pa tokom infekcije može pomoći da se glukoza usmeri ka energetskim potrebama

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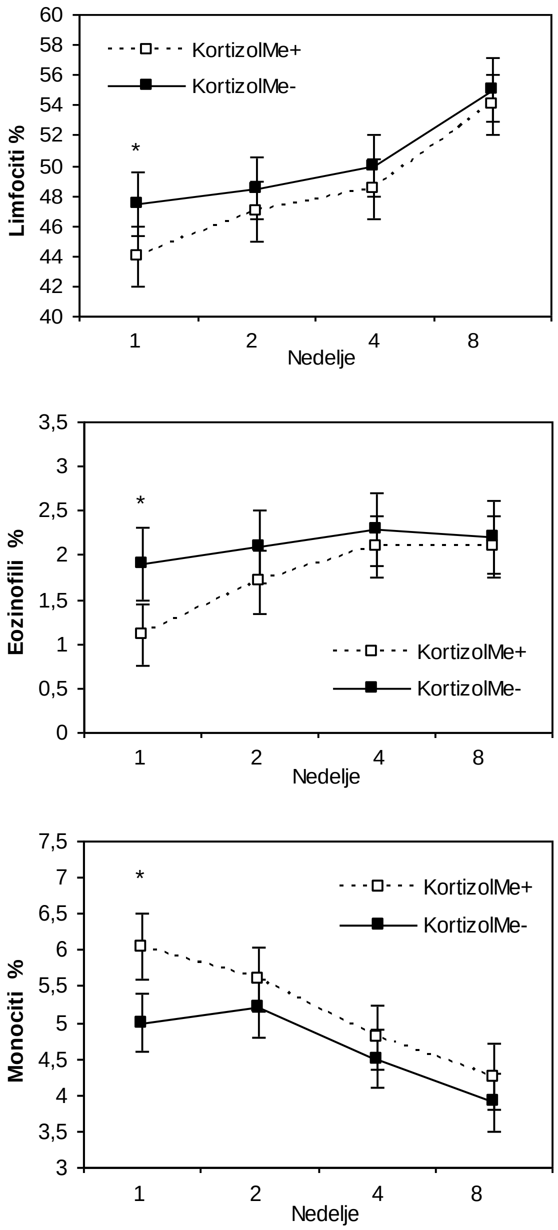
leukocita, što omogućuje da imunološke ćelije prežive (24). Smanjen unos hrane, koji je u vezi sa peripartalnim metaboličkim stresom i predhodi mu takođe kompromituje funkciju neutrofila. Kao posledica negativnog energetskog bilansa raste koncentracija NEFA, a u *in vivo* eksperimenu utvrđeno je da koncentracija NEFA preko 0,5 mmol/l smanjuje fagocitnu i oksidativnu aktivnost neutrofila (25). Ovoj koncentraciji odgovara i koncentracija medijalne vrednosti NEFA u našem modelu, što dodatno objašnjava da je vijabilnost leukocita, kao generalni pokazatelj funkcionalnog statusa umanjena u ranoj laktaciji tokom prve dve postpartalne nedelje. Tokom rane laktacije u krvi krava postoji značajno veći broj polimorfonukleara koji su u apoptozi u odnosu na srednji stadijum laktacije, dok se procenat nekrotičnih polimorfonukleara ne razlikuje u početku i na sredini laktacije (26). Ovakav nalaz se vezuje za niži procenat vijabilnih leukocita u ranoj laktaciji dobijenih u našem ogledu.

Sve navedeno ukazuje da varijabilnost hematoloških parametara zavisi od varijabilnosti pokazatelja metaboličkog stresa kod krava u periodu rane laktacije.



Grafikon 7-9. Prosečna % zasutpljenost limfocita, eozinofila i monocita kod krava klasifikovanih prema kriterijumu kortizol

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LITERATURA

1. *Cincović M., Belić B. Specijalna patološka fiziologija – praktikum. Poljoprivredni fakultet Novi Sad – Departman za veterinarsku medicinu, Novi Sad, 2021.*

ISBN 978-86-7520-611-8

2. *Belić B., Cincović M.R. Praktikum iz patološke fiziologije. Poljoprivredni fakultet Novi Sad, Departman za veterinarsku medicinu, 2012.*
3. *Radojičić B. Opšta klinička dijagnostika kod domaćih papkara. Fakultet veterinarske medicine, Beograd, 2008.*
4. *Klinkon M., Zadnik T. Dynamics of red and white blood picture in dairy cows during the periparturient period. Comparative Hematology International 1999, 9: 156-161.*
5. *Sattar A., Mirza R.H. Haematological parameters in exotic cows during gestation and lactation under subtropical conditions. Pakistan Vet J 2009, 29(3): 129-132.*
6. *Mirzadeh K.H., Tabatabei S., Bojarpour M., Mamoei M.: Comparative study of hematological parameters according strain, age, sex, physiological status and season in Iranian cattle. Journal of Animal and Veterinary Advances 2010, 9(16): 2123-2127.*
7. *Belić B., Cincović M.R., Krčmar LJ., Vidović B. Reference values and frequency distribution of hematological parameters in cows during lactation and in pregnancy. Contemporary agriculture 2011, 60, 145-151.*
8. *Belić B., Cincović M.R., Davidov I., Lako B., Potkonjak A., Stančić I. Periparturient hematological finding in dairy cows with uterus and udder inflammation. Contemporary agriculture 2012, 61(1-2): 112-118.*
9. *Belić B., Cincović M.R., Stojanović D., Kovačević Z., Vidović B. Morphology of erythrocyte and ketosis in dairy cows with different body condition. Contemporary agriculture 2010, 59, 306-311.*
10. *Cincović M.R., Belić B., Radojičić B., Hristov S., Đoković R. Influence of lipolysis and ketogenesis to metabolic and hematological parameters in dairy cows during periparturient period. Acta veterinaria (Beograd) 2012, 62(4): 429-444.*
11. *Kovačević Filipović M. Osobenosti krvne slike goveda tokom njihovog rasta i razvoja. Zbornik Poremećaj zdravlja krava u puerperijumu i zdravstveni status teladi, 85-100, 2007.*
12. *Davis A.K., Maney D.L., Maerz1 J.C. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Functional Ecology 2008, 22, 760–772.*
13. *Tornquist S.J., Rigas J. Interpretation of Ruminant Leukocyte Responses. In: Schalms veterinary hematology. 6th edition, Wiel-Blackwell, 307-313, 2010.*
14. *Lynch E.M., Earley B., McGee M., Doyle S. Characterisation of physiological and immunological responses in beef cowsto abrupt weaning and subsequent housing. BMC Veterinary Research 2010, 6:37.*
15. *Bertoni G., Trevisi E., Ferrari A., Archetti I. Preliminary studies on compatibility between high yield levels and the well-being of dairy cows. Vet Res Commun 2003, 27(Suppl 1): 639–641.*
16. *George J.W., Snipes J., Lane V.M. Comparison of bovine hematology reference intervals from 1957 to 2006. Vet Clin2010, 39 (2): 138–148.*
17. *Belić B., Cincović M.R., Stevančević M., Toholj B. Parameters of negative energy balance (NEFA, BHB and glucose) and neutrophil to lympocyte ratio in cows after calving – compariosn of two indicators of stress. 12th Middle European Buiatric Congress, Pula, Croatia, May 18-22, 281-284, 2011.*
18. *Thanasak J., Jorritsma R., Hoek A., Noordhuizen J., Rutten V., Müller K.E. The effects of a single injection of dexamethasone-21-isonicotinate on the lymphocyte functions of dairy cows at two weeks post partum. Vet. Res. 2004, 35: 103–112.*
19. *Latimer K.S., Prasse K.W. Leukocytes. In: Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology. 4th ed. Ames, IA: Iowa State Press, 46–79, 2003.*
20. *Rafia S., Taghipour-Bazargani T., Khaki Z., Bokaie S., Sattari Tabrizi S. Effect of body condition score on dynamics of hemogram in periparturient Holstein cows. Comparative Clinical Pathology 2012, 21(5): 933-943.*
21. *Morris D.G., Waters S.M., McCarthy S.D., Patton J., Earley B., Fitzpatrick R., et al. Pleiotropic effects of negative energy balance in the postpartum dairy cow on splenic gene expression: repercussions for innate and adaptive immunity. Physiol. Genomics 2009, 39: 28-37.*
22. *Galvão K.N., Flaminio M.J.B.F., Brittin S.B., Sper R., Fraga M., Caixeta L., Ricci A., Guard C.L., Butler W.R., Gilbert R.O. Association between uterine disease and indicators of neutrophil and systemic energy status in lactating Holstein cows. J Dairy Sci 2010, 93: 2926-2937.*
23. *Nielsen L., Røntved C.M., Nielsen M.O., Norup L.R., Ingvartsen K.L. Short Communication: Leukocytes from heifers at different ages express insulin and insulin-like growth factor-1 (IGF-1) receptors. Domest. Anim. Endocrin. 2003, 25: 231-238.*
24. *Maratou E., Dimitriadis G., Kollias A., Boutati E., Lambadiari V., Mitrou P., Raptis S.A. Glucose transporter expression on the plasma membrane of resting and activated white blood cells. Eur J Clin Invest 2007, 37(4): 282-290.*
25. *Scalia D., Lacetera N., Bernabucci U., Demeyere K., Duchateau L., Burvenich C. In Vitro Effects of Nonesterified Fatty Acids on Bovine Neutrophils Oxidative Burst and Viability. J.Dairy Sci. 2006, 89:147-154.*
26. *Van Oostveldt K., Vangroenweghe F., Dosogne H., Burvenich C. Apoptosis and necrosis of blood and milk polymorphonuclear leukocytes in early and midlactating healthy cows. Vet. Res. 2001, 32: 617–622.*

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HEAT STRESS IMPACT ON SHEEP PRODUCTION

UTICAJ TOPLOTNOG STRESA NA PROIZVODNJU OVACA

Aleksandar Čukić[a*], Marko Cincović[b], Radojica Djoković[c], Simeon Rakonjac[c], Milun Petrović[c] and Miloš Ž. Petrović[c]

[a] Faculty of Agriculture, University of Priština in Kosovska Mitrovica, Kopaonička bb, 38219 Lešak, Serbia;
[b] Faculty of Agriculture, University of Novi Sad, Square Dositeja Obradovića 8, 21000 Novi Sad, Serbia;
[c] Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, 32000 Čačak, Serbia.
[*]Corresponding author: aleksandar.cukic@pr.ac.rs

A.Č. https://orcid.org/0000-0001-9063-3431, M.C. https://orcid.org/0000-0002-5683-8983, R.Dj. https://orcid.org/0000-0003-0900-3227, S.R. https://orcid.org/0000-0002-1232-1755, M.P. https://orcid.org/0000-0001-8187-0785, M.Ž.P. https://orcid.org/0000-0002-4707-6796.

ABSTRACT

Research on the impact of heat stress on animals has mainly been related to cattle, while sheep have been neglected and the impact of heat stress on sheep production is still insufficiently researched. There are numerous stressors related to the procedures and methods of breeding sheep in barns and pastures among them ambient temperature is the most important variable because its effect is exacerbated in the presence of high humidity. Thermal indices are useful for assessing the influence of weather parameters in a certain agroecological area, of which the temperature-humidity index proved to be the best thermal index for assessing the harmful effect of heat stress on the productive performance of animals. Sheep have good adaptability and they are resistant to harsh environmental conditions, still in addition to a certain tolerance to heat stress, high temperatures can negatively affect sheep, which most often leads to dehydration, reduced appetite, reduced milk production and increased risk of disease. Mechanisms that help sheep to survive the challenge of heat stress include morphological, behavioural, physiological, blood biochemistry and genetic bases of adaptation. Sheep can combat heat stress by seeking shade, drinking enough water, and properly ventilating the barn. Increasing the productivity of sheep by adapting various management strategies including housing and animal management and climate monitoring may enhance production capacity of the herd. Therefore, heat stress has a negative effect on sheep, temperatures will increase year by year, and therefore it is necessary to investigate the relationship between sheep production and heat stress in time, to improve sheep farming and make life easier in the days ahead.

Keywords: climate change, temperature-humidity index, sheep production, adaptation, sheep management

SAŽETAK

Istraživanja uticaja toplotnog stresa na životinje su se uglavnom odnosila na goveda, pri čemu su ovce ostale zapostavljene i još uvek je nedovoljno istražen uticaj toplotnog stresa na ovčarsku proizvodnju. Brojni su stresori vezani za postupke i metode uzgoja ovaca u štalama i pašnjacima, među kojima je temperatura okoline najvažnija varijabla jer se njeno dejstvo pogoršava u prisustvu visoke vlažnosti. Termalni indeksi su korisni za procenu uticaja vremenskih parametara na određenom agroekološkom području, od kojih se indeks temperature i vlažnosti pokazao kao najbolji termalni indeks za procenu štetnog uticaja toplotnog stresa na produktivne performanse životinja. Ovce poseduju dobru prilagodljivost i otporne su na oštre uslove životne sredine, ali i pored određene tolerancije na toplotni stres, visoke temperature mogu negativno uticati na ovce, što najčešće dovodi do dehidracije, smanjenog apetita, smanjenja proizvodnje mleka i povećanja rizika od bolesti. Mehanizmi koji pomažu ovcama da prežive izazov toplotnog stresa uključuju morfološke, bihejvioralne, fiziološke, biohemijske i genetske osnove adaptacije. Ovce se mogu boriti sa

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toplotnim stresom tražeći hladovinu, pijući dovoljno vode i pravilnom ventilacijom staje. Povećanje produktivnosti ovaca prilagođavanjem različitih strategija upravljanja uključujući smeštaj i upravljanje životinjama i praćenje klime može poboljšati proizvodni kapacitet stada. Dakle, toplotni stres ima negativan uticaj na ovce, temperature će iz godine u godinu sve više rasti i zato je neophodno na vreme istražiti odnose između ovčarske proizvodnje i toplotnog stresa, unaprediti ovčarstvo i olakšati život u danima koji nam dolaze.

Ključne reči: klimatske promene, indeks temperature i vlažnosti, ovčarska proizvodnja, adaptacija, menadžment ovaca

1. Introduction

Climate change can have a significant impact on animals. Increases in temperature, changes in rainfall and other factors can affect their feeding, reproduction, migration and survival. Animals must adapt to new conditions in order to survive. The impact of climate change on livestock production will depend on the magnitude and nature of the changes and can be mediated through direct effects on animals and indirect impacts on their environment; for example, through the quantity and quality of food. Among the environmental variables that affect animals, heat stress is a major factor that makes animal production challenging in many parts of the world. Heat stress is one of the most harmful factors that contribute to reduced growth, production, reproduction, quantity and quality of milk, as well as natural immunity, making animals more vulnerable to disease and even death. However, small ruminants have successfully adapted to this extreme environment and possess some unique adaptive traits due to behavioral, morphological, physiological and mostly genetic bases. However, complete information is lacking on how these animals can adapt and survive in new and changing environments (1).

In general, sheep have good adaptability and they are resistant to harsh environmental conditions. Sheep adapt to extreme weather conditions through behavioral, morphological, physiological and mostly genetic bases (2,3). However, physiological and behavioral changes in response to hot environments affect the production of small ruminants (4). In general, sheep are raised on pastures in relatively large groups that rely on low inputs in terms of feed, water and labour, and possess a high thermotolerance compared to large ruminants. Globally, the number of sheep is increasing the most compared to other livestock, considering the problem of global warming and climate change, sheep can be crucial for maintaining the production of animal proteins, which will ensure the existence of mankind. Research on the impact of heat stress on animals has mainly been related to cattle, while sheep have been neglected and the impact of heat stress on sheep production is still insufficiently researched. In addition to a certain tolerance to heat stress, high temperatures can negatively affect sheep, which most often leads to dehydration, reduced appetite, reduced milk production and increased risk of disease. Sheep can combat heat stress by seeking shade, drinking enough water, and properly ventilating the barn. Therefore, heat stress has a negative effect on sheep, temperatures will increase year by year, and therefore it is necessary to investigate the relationship between sheep production and heat stress in time, to improve sheep farming and make life easier in the days ahead.

This review paper aims to provide an insight into the state of sheep production in conditions of heat stress, look at different mechanisms of sheep adaptation and strategies for improving sheep production in order to successfully deal with the global problem called heat stress.

2. CLIMATE CHANGE AND LIVESTOCK PRODUCTION

The climate has changed several times in the geological past of the planet, which was influenced by various factors, from the composition of the atmosphere, through volcanic eruptions, reactions in the sun, etc. However, since the agricultural revolution 10,000 years ago, climatic conditions have not changed much. However, in the second half of the last century, it became clear that the climatic conditions were beginning to change too quickly. Since the beginning of the industrial revolution, in 150 years the average temperature on earth has increased by 1°C (Figure 1.), which causes numerous changes in the climate.

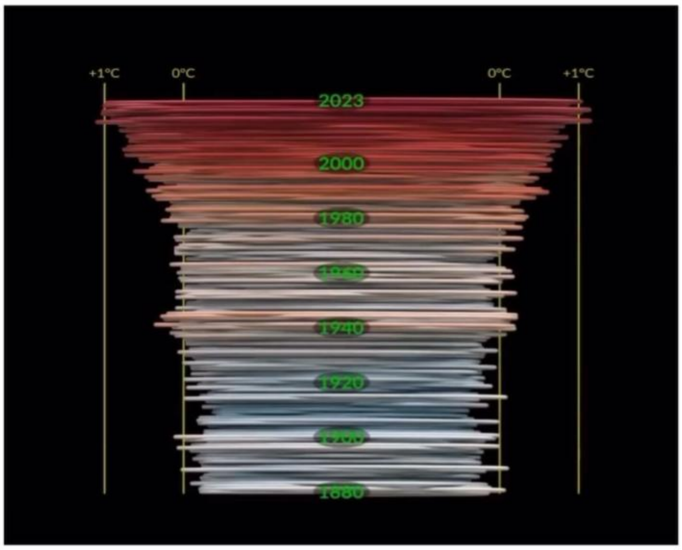

Figure 1. Changes in temperature since the beginning of the industrial revolution.

Scientists have proven that this does not happen naturally, but that humans are responsible for the increase in temperature. As much as 80% comes from the burning of fossil fuels, coal, oil and gas for the production of electricity, industry, heating and transport. Livestock contributes to almost 18% of total anthropogenic greenhouse gas emissions. If we do not stop burning fossil fuels, our planet will continue to heat up, which in the future will cause more frequent and powerful heat waves, more frequent droughts, melting of polar caps and glaciers, then rising sea levels, stronger and more destructive storms and more frequent floods. The confounding of greenhouse gas emissions by anthropogenic activities on climate change has fueled global research efforts regarding the contribution of livestock to global warming. Further efforts are needed to comprehensively assess the multiple impacts of climate change on different ecosystem components to gain a thorough understanding of this topic.

Climate change is defined as a long-term imbalance in the normal weather conditions such as temperature, wind and precipitation characteristics of a particular region and is likely to be one of the major challenges facing humanity during the current century (5). Livestock production plays an important role in the global economy. The effects of climate change are not limited to crop production, but also affect livestock production. Therefore, livestock-based food security is at risk in many parts of the world. Livestock react to environmental changes by changing their phenotypic and physiological characteristics. Therefore, the survival of an animal often depends on its ability to cope with or adapt to existing conditions. Therefore, in order to sustain livestock production in a climate-induced environment, animals must be genetically fit and have the ability to survive in diverse environments. (6).

2.1. TYPES OF ENVIRONMENTAL STRESS IN SHEEP

There are numerous stressors related to the procedures and methods of breeding sheep in barns and pastures. The most significant stressors on farms and pastures come from unfavorable housing conditions, improper handling, veterinary and zootechnical measures and procedures (treatment, vaccinations, blood tests, operations, marking, weaning, grouping, clipping, tail trimming, hoof care), inadequate climatic conditions (extremes of heat and cold) and nutrition (7). The effect of these stressors is manifested in all life periods of sheep, but there are periods when they are subjected to additional loads and the animals are more susceptible to these stressors, such as partus, soon after birth, juvenile period, puberty, estrus, advanced pregnancy and puerperal period. Some stressors may be qualitatively physical components, such as

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climate, while others involve certain procedures in handling procedures, such as manipulation of animals, introduction of new diet, new environment or new animals in the herd, etc. (7) Components that can cause stress in sheep are ambient temperature, humidity, air flow, solar radiation, etc., among which ambient temperature is the most important (8). In the livestock environment, ambient temperature is the most important variable because its effect is exacerbated in the presence of high humidity. Heat stress is defined as any combination of environmental parameters that create conditions that are higher than the temperature range of the animal's thermoneutral zone (TNZ). (9).

2.2. SHEEP PRODUCTION IN THE CHANGING CLIMATE SCENARIO

Climate change affects sheep production both directly and indirectly. The production losses incurred for climate change in sheep could be attributed to the low pastures, low water availability and disease outbreaks (10). In general, sheep are raised in extensive farming systems, with a small percentage of intensive sheep farming. Much of the production is in extensive pastures where inputs are low and feed production and water supply vary with seasonal climate. Intensive ruminant production is more vulnerable to welfare and health impacts of climate changes through heat stress and there is a threat to both intensive and extensive systems of new or expanded exposure to pests and disease (11). The adaptive profiles of sheep have been well studied in different farming systems (12,13). Heat stress affects performance and productivity of small ruminants in all phases of production. The degree to which these stress impacts on productivity will differ between the agroecological regions and between production systems (14). Sheep possess superior ability to convert more fibrous and low-quality feed to meat than cattle. Native sheep breeds of arid and semiarid regions have higher adaptability to harsh environmental conditions compared to exotic breeds (15). Hence, appropriate breed selection is an effective tool to sustain production in the changing climatic conditions (16).

Even though sheep show higher adaptation to harsh environment, the fast-changing climate could affect the sustainable production through low feed intake, variation in energy and mineral metabolism, alterations in water and protein balances, etc. (8,17). The key constraints such as thermal-, nutritional- and water-related stresses reduce productivity of the sheep in hot and dry regions (18, 19). In addition, the indirect effects of increased incidence of disease and parasite infection and reduced pasture availability also contribute to additional stress and produce decreased wool, milk and meat production in sheep (20). Since most of the sheep population are owned by poor sections of the society, loss of production may lead to severe poverty in rural areas (15). Loss of production and reproduction in sheep during heat exposure can be partially explained by lower feed intake, but is also influenced by altered endocrine and metabolic status. Performance strongly affected by high temperatures includes a range of neuroendocrinological, physiological and behavioral responses, which affect the balance of animal functions (8). Such responses can promote alterations in the level of blood metabolites and metabolic hormones (21, 22, 23). Based on stated, among the various climatic factors, heat stress appears to be the main intriguing factor hindering modern sheep production.

3. MEASUREMENTS OF SEVERITY OF HEAT STRESS IN SHEEP

Heat stress is defined as a condition in which the organism is exposed to ambient temperatures that are outside the biological optimum, which leads to the amount of heat produced in the body being greater than the amount consumed. In a state of heat stress, energy is spent on cooling, i.e. maintaining homeothermy, instead of maintaining productive traits. The animals perform efficiently in their thermoneutral zone. The zone above or below the critical temperature constrains the animal’s productivity as they undergo stress. Exposure of sheep to severe heat stress demands the metabolic system of body to dissipate the excess heat by increasing respiratory rate, sweat rate, rectal temperature and pulse rate (15). Various thermal indices like temperature-humidity index (THI), black globe-humidity index (BGHI), equivalent temperature index (ETI), environmental stress index (ESI), heat load index (HLI) and respiratory rate predictor (PRR) have been assessed for livestock considering the local weather condition including all cardinal weather parameters based on physiological adaptability (24). BGHI and THI is by far the best assessment tool to evaluate the effect of heat stress.

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Black global humidity index (BGHI) refers to daily temperatures, relative humidity, degree of radiation and wind speed. BGHI is expressed as total solar radiation as a function of direct solar rays and the angle at which they fall on a certain surface (25). Temperature and humidity index (THI) is the simplest approach, which combines ambient temperature and relative humidity to assess the response of livestock productivity as a function of climate (26). As temperature and humidity are often readily collected, the minimal inputs make THI an easy tool for retrospective studies in most regions (27). The most reliable THI values for assessing the thermal load of sheep are described by Marai et al. (28): normal < 27.8, moderate 27.8–28.8, severe 28.9–29.9 and very severe (emergency) ≥ 30.0. It is important to recognise that thermal stress is not initiated by ambient temperature alone, and that there are a variety of cardinal weather variables that can assist in the assessment or prediction of high heat load (17).

It is assumed that an animal's core temperature reflects the temperature of the main internal organs such as the heart, brain and viscera (29). Rectal temperature has long been used to evaluate core temperature and to quantify the heat stress response in livestock (30). Another common location for measuring core temperature is the vagina, which is well insulated and characterised by thermal gradients (31). Over the past decade, intra-ruminal insertion of temperature sensors has emerged as a non-invasive alternative to the surgical implantation of devices (32), but research shows the potential impact of hyperthermia on rumen function in sheep (33). Measuring the heat emitted by the body is becoming more and more popular, and the thermal imaging camera has found its wide application. The thermal imaging camera represents a modern and non-invasive assessment of thermal status. Thermo-graphic images can be used to demonstrate an increase in body temperature and changes in blood flow related to stressful environmental conditions such as high heat load (34). In our previous research (35) carried out on sheep during the summer, the body surface temperature was measured by collecting images in different parts of the body-thermal window: eye temperature (ET), nose (NT), front leg (LT) and abdomen temperature (AT). Results shown that the abdomen and legs are good thermal windows because LT and AT are good summative responses to external ambient THI and internal metabolic changes in sheep under heat stress, with significant correlation with almost all examined blood parameters and with the THI, so it could be a suitable method in the non-invasive assessment of stress load in sheep. A range of subcutaneous microchips and other implantable devices are also being developed for the continuous measurement of body temperature in livestock (36, 37). However, all require surgical procedures for implantation. In addition to their invasive nature, subcutaneous temperature is influenced by environmental conditions and physiological state as this directly effects the flow of blood to the skin (38). The development of predictive model software which facilitates ease of data collection, management and integration would be a key milestone in adopting this technology at an industry level. The associated costs of these technologies and their comparable relationship to core temperature is likely to be a key determinant in which technologies provide the most helpful information in preventing and managing heat stress (27).

4. DIFFERENT MECHANISMS OF SHEEP ADAPTATION

Among species, sheep and goats are considered less sensitive to heat stress than cattle (39, 40). In brief, the adaptation is the level of tolerance to survive and reproduce under extreme living conditions (41). Sheep are very rustic animals that can cope with such an environment. However, full information on how these animals can adapt and survive to the novel and transforming environments are lacking. The adaptation of sheep breeds to different locations/climates depends upon temperature, humidity, vegetation and wool cover and resistance/susceptibility to various diseases. Sheep breeds can tolerate a wide range of climate and convert poor-quality forage into quality animal protein. These characters favour their rearing under extensive system among poor rural people in harsh climate (42). There are several phenotypic and genotypic characters which impart the adaptive potential to an animal, thereby allowing it to cope with harsh conditions. Basically, adaptation involves morphological, behavioral and genetic capacity of the animal for change (6). The adaptive process can be expanded to include: 1) morphological, 2) behavioural, 3) physiological, 4) blood biochemistry and 5) genetic bases of adaptation (Figure 2). These mechanisms help sheep to survive the heat stress

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challenges. Morphological adaptations are physical changes that occur over many generations of animals that enhance its fitness in a given environment. Body size and shape, coat and skin color, hair type, and fat storage are among the main morphological adaptation in sheep (43). Body size and shape are the most dominant morphological characteristics influencing the thermoregulatory mechanisms of farm animals in extremely hot environments. The authors stated that animals with larger body size have lower metabolic rate than that of smaller animals and gain heat at a slower rate (44, 45). The coat and skin color characteristics of sheep that have evolved in tropical and desert areas are different from those that evolved in temperate climates. For instance, the loose, open fleece of hair and wool of Awassi sheep enhances heat loss via convection (46). Wool is a natural indicator of short-term stress exposure within the sheep's natural environment. Wool fibres reduce in microns at the point of stress caused by individual events or through a combination of factors (nutrition, disease or pregnancy) (9). Most of the time, fat tails and rump fat are considered as an adaptive response of animals to extreme environments and are used as a valuable energy reserve for the animal during migration and winter. About 25% of the world's sheep population comprises fat-tailed breeds (47).

Behavioral adaptation is recognized as the first and foremost response adopted by animals to reduce heat load (48). One of the most quick and profound behavioral changes seen in heat stressed animals is shade seeking. The stressed animals attempt to ameliorate the negative effects of direct heat load by using shade whenever they can access to it (6). When animals are exposed to high temperatures, reduction of feed intake will occur. Reducing feed intake is a method of adaptation to decrease heat production in the warm environment as the heat increment of feeding is an important source of heat production in ruminants (49). Generally, heat stressed animals tend to spend more time standing so that they can reorient themselves in different directions to avoid direct solar radiation and ground radiation. In addition, the standing position also obstructs the conductive heat transfer into the animal body due to the presence of a layer of air adjacent to the skin, and also facilitates the dissipation of body heat load to the surroundings by increasing the amount of skin exposed to air flow or wind (6). Higher drinking frequency and increased water intake were reported for various livestock species during summer (48). Breeds adapted to desert regions compensate higher water loss during periods of high heat load by concentrating urine (50).

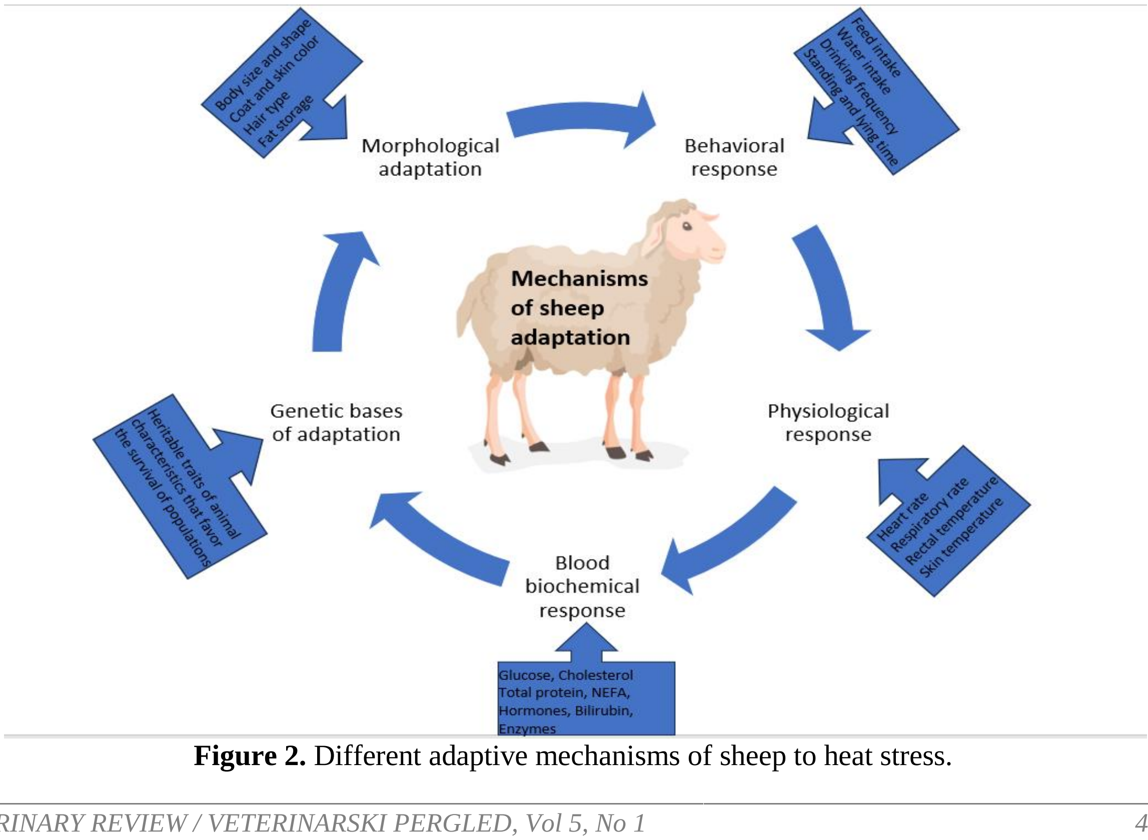

Figure 2. Different adaptive mechanisms of sheep to heat stress.

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Animals possess a variety of physiological adaptation mechanisms in the reduction of heat load. Change in heart rate, respiration rate, skin temperature and rectal temperature are the key parameters that indicate the mechanism of physiological adaptation in sheep. Rectal temperature is a good index of body temperature even though there is a considerable variation in several parts of the body score at different times of the day. In heat-stressed environments, respiratory rate is the first thermoregulation mechanism used by sheep to help them maintain their body temperature (51). Exposure of sheep to hot environment also increased skin temperature. This higher skin temperature could be directly attributed to the vasodilatation of skin capillary bed to enhance the blood flow to the skin periphery for facilitating heat transfer to the surroundings (48). When the physiological mechanism fails to alleviate the effect of heat load, the body temperature may increase to a point at which animal well-being is compromised. Body temperature is a good measure of heat tolerance in animals, as it represents the result of all heat gain and heat loss processes in the body (51).

Blood metabolite concentrations are one of the main regulators of animal adaptation to HS challenges. HS significantly changes glucose homeostasis in animals, the results of the effect of high ambient temperature on the blood glucose (GLU) content of sheep are conflicting. Maraii et al. (52) found in Ossimi sheep that blood GLU levels were significantly higher in summer than in winter. Some other studies showed that blood GLU decreased significantly with different percentages (in Chios sheep and crossbred Chios x Ossimi) (53). Srikandakumar et al. (54) examined the effect of HS on the metabolism of Omani and Merino sheep, where they observed that HS increased GLU in Merino but decreased it in Omani sheep. Which means that a variety of variables can affect GLU metabolism, including breed, age, breeding, hormones, physiological state (i.e. pregnancy, lactation, malnutrition and disease), and the most important nutritional influences. Total lipid concentration significantly decreases in ruminants with prolonged exposure to high ambient temperature, especially cholesterol values. The concentration of total proteins increases in HS. Salem et al. (53) observed that serum total proteins levels were higher during hot summer than winter in Chios lambs and crossbred Chios x Ossimi in Upper Egypt. Another important metabolic regulator is non-esterified fatty acids (NEFA) in plasma and serum (55). Endocrine responses are one of the main regulators of animal adaptation to HS challenges. Cortisol, the main glucocorticoid, is mainly produced in the adrenal cortex, is considered an important stress marker, and participates in various bodily functions, including immune responses and protein, carbohydrate, and fat metabolism (56). Under conditions of HS, it is common to observe a decrease in the levels of thyroid hormones T3 and T4, both responsible for mediating animal metabolism, as a mechanism to reduce metabolic heat production (57). Indicators of the functional state of the liver are the concentration of bilirubin and the activity of enzymes in the blood, which can also indicate metabolic stress. The effects of HS on blood metabolite concentrations vary widely across studies, making it difficult to explain the metabolic adaptations made by sheep to survive and adapt to hot climates (35).

The adaptive capability of sheep is determined by their genetic potential. The research findings from one type of sheep may not be necessarily applicable to the other kind of sheep. Another limitation is that most of the studies were for short term and conducted in laboratories located in the temperate zone on British/Merino breeds (15). Adaptation in terms of genetics refers to the heritable traits of animal characteristics that favor the survival of populations (58). Adaptation traits are usually characterized by low heritability. Genetic variation in a population provides flexibility to adapt to the changing environment and it is crucial for the survival of the population over time (51). The genetic basis of heat adaptation is poorly understood. Evidence from different researchers indicated that the role of genetics in determining an individual's capability to adapt to the stressed environment is very complicated. As a result, in the cellular energy, mitochondria play a central role as a facilitator of energy metabolism (59). Genome and genomic studies help to investigate thermo-tolerance genes and genomic regions that play a significant role for regulation of body temperature in small ruminants (60).

5. MANAGEMENT STRATEGIES TO COUNTER HEAT STRESS IN SHEEP

Substantially increasing the productivity of these animals by adapting various management strategies including housing and animal management and climate monitoring may enhance production capacity of the flock, and hence better output can be expected from the animals in terms of meat, wool, milk and number of offspring (15). Simultaneously efforts are also needed to identify the most appropriate strategies for a particular location and the other factors that influence the genetic merits of animals that question their survival in a particular environment (61). The management strategies can be categorized as: housing management, nutritional modifications, genetics and breeding, and health management (Figure 3).

The housing must reduce the quantum of stress during adverse environmental condition and provide a suitable environment for the animals to survive and produce optimally. Under housing management, the type of shelter, availability of shade, ventilation and light availability inside the shed are considered as the major factors affecting the productivity of the animal. At times of heat stress during summer, water and shade availability around the shed are crucial factors (15). Supplementation of sheep diets with vitamin and mineral, especially with Vit E and Se at supranutritional levels successfully reduced heat-induced oxidative stress. Chauhan et al. (62) study suggests that supranutritional levels of antioxidants (specifically Vit E and Se) are needed to alleviate the negative effects of heat stress on redox homeostasis in sheep. Also, seasonal specific feeding is one of the factors to be considered in nutrition management. Apart from these factors, selection of breed is given primary importance as sheep farming nowadays is production oriented. So selecting the genetically superior breeds for a given trait like meat, wool, milk and adaptation capability is advisable to get desirable income (30). A key component of adaptation, however, is the genetic ability of an organism to survive under stressful conditions. Genetic selection for heat tolerance would provide a sustainable means of augmenting feeding and/or housing modifications (63). In a review of such strategies, Gaughan (64) suggests to identify existing local breeds that are already adapted to production under environmental stresses and to allocate stress-adaptive genes in these breeds would provide a way forward. Subsequently, selection signatures for thermotolerance can be identified through functional genomics and productive breeds improved through cross-breeding with resilient genotypes and incorporation of stress-tolerant genes (65). Health and also disease status has to be monitored regularly. Animal management itself is another important factor to be considered. Way of handling the animal that include transport, shearing and sorting imparts a major effect on the productivity of the animal.

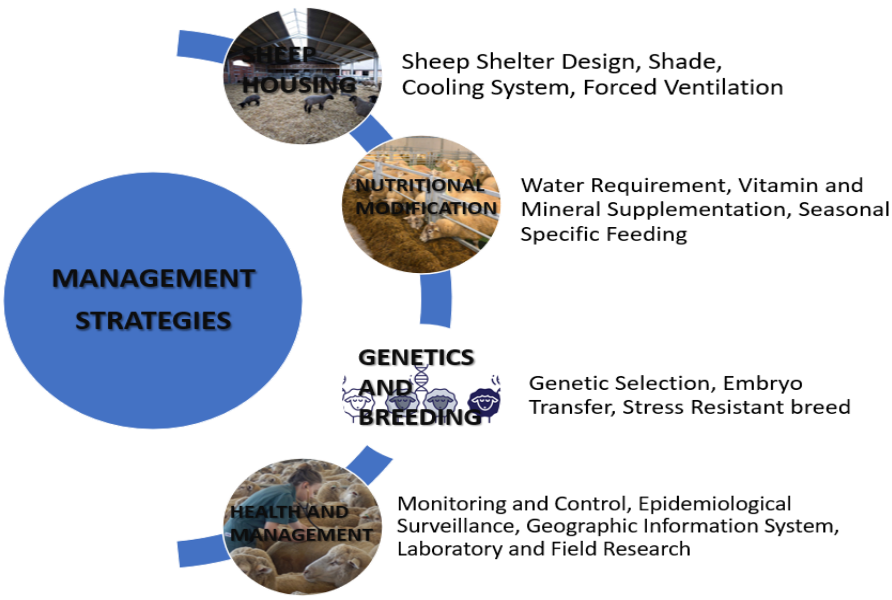

Figure 3. Management strategies to improve sheep production

6. CONCLUSIONS

Ruminant production systems will need to adapt as the climate continues to change, in line with the expectation that they will also contribute to the goals of reducing greenhouse gases and minimizing other negative environmental impacts. The projected increase in extreme weather events and changes in the availability, composition and quality of food, as well as in animal nutrition will affect the availability of animal products and the food supply. These effects are likely to be greatest in developing countries where population and food demand are greatest. Given that sheep are more resistant to heat stress than cattle, it is inevitable that they will represent the main source of animal protein in the coming days. Accordingly, it is necessary to understand to what extent and in what way heat stress affects sheep production, examine adaptive mechanisms in detail and work on strategies to improve sheep production. This review will provide researchers with a basis for predicting when sheep is under heat stress. The only conditions modern humans have ever known so far, are changing, and changing fast. What we do now, and in the next few years will profoundly affect the next few thousand years.

REFERENCES

1. *Berihulay, H.; Abied, A.; He, X.; Jiang, L.; Ma, Y. Adaptation Mechanisms of Small Ruminants to Environmental Heat Stress. Animals 2019, 9 (75), 1–9. https://doi.org/10.3390/ani9030075.*
2. *Seixas, L.; De Melo, C.B.; Tanure, C.B.; Peripolli, V. Heat Tolerance in Brazilian Hair Sheep. Asian-Australas. J. Anim. Sci. 2017, 30, 593–601.*
3. *José, C.; Manuel, A.; Pereira, F.; De Mira, A.; Morita, L.; Antonio, E.; Titto, L. Thermoregulatory Response in Hair Sheep and Shorn Wool Sheep Cristiane Gonc. Small Rumin. Res. 2016, 144, 341–345.*
4. *Li, F.K.; Yang, Y.; Jenna, K.; Xia, C.H.; Lv, S.J.; Wei, W.H. Effect of Heat Stress on the Behavioral and Physiological Patterns of Small-Tail Han Sheep Housed Indoors. Trop. Anim. Health Prod. 2018, 50, 1893–1901. https://doi.org/10.1007/s11250-018-1642-3*
5. *Bernabucci U.; Lacetera N.; Baumgard L.; Rhoads R.; Ronchi B.; Nardone A. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal. 2010, 4(07), 1167-1183. DOI: 10.1017/s175173111000090x*
6. *Sejian, V.; Bhatta, R.; Gaughan, J. B.; Dunshea, F. R. & Lacetera, N. Review: Adaptation of animals to heat stress. Animal 2018, 1–14. doi:10.1017/s1751731118001945.*
7. *Hristov, S.; Maksimović, N.; Stanković, B.; Žujović, M.; Pantelić, V.; Stanišić, N. & Zlatanović, Z. The most significant stressors in intensive sheep production. Biotechnology in Animal Husbandry 2012, 28(4), 649-658. http://dx.doi.org/10.2298/BAH1204649H*
8. *Marai M.F.I.; El-Darawany A.A.; Fadie A.; Abdel-Hafez M.A.M. Physiological traits as affected by heat stress in sheep—A review. Small Rumin. Res. 2007, 71, 1–12.*
9. *Sawyer, G., & Narayan, E. J. A review on the influence of climate change on sheep reproduction. Comparative endocrinology of animals 2019, 10.*
10. *Sejian V.; Bahadur S. and Naqvi S.M.K. Effect of nutritional restriction on growth, adaptation physiology and estrous responses in Malpura ewes. Anim Biol 2014, 64:189–205.*
11. *Henry, B. K.; Eckard, R. J.; Beauchemin, K. A. Review: Adaptation of Ruminant Livestock Production Systems to Climate Changes. Animal, 2018, 12 (s2), S445–S456. https://doi.org/10.1017/S1751731118001301.*
12. *Kochewad, S. A.; Traghunandan; Rao, K.; Reddy, K. K.; Kumari, N.; Ramana, D.; Kumar, S.; Meena. L.R.; Singh, S. P. Reproductive Performance and Body Condition Score of Deccani Sheep during Various Physiological Stages in Different Farming Systems. Indian J. Anim. Sci. 2018, 88 (12), 1379–1382. https://www.researchgate.net/publication/330310367*
13. *Kochewad, S. A.; Raghunandan, T.; Sarjan Rao, K.; Kondal Reddy, K.; Nalini Kumari, N.; Ramana, D.B.V.; et al. Productive Performance, Body Condition Score and Carcass Characteristics of Deccani Lambs Reared under Different Farming Systems. Indian J Anim Res 2018, 52 (3), 444–448. https://doi.org/10.18805/ijar.B-3478.*
14. *Shekhawat, I.; Pareek, A. A Review: Growth and Physiological Adaptability of Sheep to Heat Stress under Semi-Arid Environment. Int. j. emerg. trends sci. technol. 2015, 02 (09), 3188–3198. https://doi.org/10.18535/ijetst/v2i9.09.*
15. *Sejian, V.; Bhatta, R.; Gaughan, J.; Malik, P.K.; Naqvi, S.M.K. and Lal, R. 2017. Adapting Sheep Production to Climate Change. In: Sejian, V., Bhatta, R., Gaughan, J., Malik, P., Naqvi, S., Lal, R. (eds) Sheep Production Adapting to Climate Change. Springer, Singapore. https://doi.org/10.1007/978-981-10-4714-5_1*
16. *Iniguez L. Characterization of small ruminant breeds in West Asia and North Africa, vol 1. ICARDA 2005, Aleppo*
17. *Finocchiaro R.; Van Kaam J.B.C.H.M.; Portolano B.; Misztal I. Effect of heat stress on production of Mediterranean dairy sheep. J Dairy Sci 2005, 88(5), 1855–1864. https://doi.org/10.3168/jds.S0022-0302(05)72860-5*

18. *Kandemir C.; Kosum N.; Taskin T. Effects of heat stress on physiological traits in sheep. Maced J Anim Sci 2013, 3:25–29.*
19. *Sejian V. Climate change: impact on production and reproduction, adaptation mechanisms and mitigation strategies in small ruminants: a review. Indian J Small Rumin 2013, 19(1):1–21.*
20. *Singh S.K.; Meena H.R.; Kolekar D.V.; Singh Y.P.; Climate change impacts on livestock and adaptation strategies to sustain livestock production. J Vet Adv 2012, 2(7):407–412.*
21. *Marai, I.; El-Darawany, A.; Fadiel, A.; Abdel-Hafez, M. Reproductive performance traits as affected by heat stress and its alleviation in sheep. Trop. Subtrop. Agroecosyst. 2008, 8, 209–234.*
22. *Sejian, V.; Maurya, V.P.; Naqvi, S.M. Adaptive capability as indicated by endocrine and biochemical responses of Malpura ewes subjected to combined stresses (thermal and nutritional) in a semi-arid tropical environment. Int. J. Biometeorol., 2010, 561 54(6), 653–661. doi: 10.1007/s00484-010-0341-1.*
23. *Macias-Cruz, U.; Alvarez-Valenzuela, F.D.; Correa-Calderon, A.; Diaz-Molina, R.; Mellado, M.; Meza-Herrera, C.A.; Thermoregulation of nutrient-restricted hair ewes subjected to heat stress during late pregnancy. J. Therm. Biol., 2013, 38, 1–9. 564 doi: 10.1016/j.jtherbio.2012.09.002.*
24. *Hahn G.L.; Mader T.L.; Eigenberg R.A. Perspective on development of thermal indices for animal studies and management. EAAP Technic Ser 2003, 7:31–44.*
25. *Buffington, D.E.; Collier, R.J.; Canton, G.H. Shade man- cattle agement systems to reduce heat stress for dairy cows in hot humid climates. Trans. Am. Soc. Agric. Eng. 1983, 26, 1798–1803.*
26. *Lallo, C.; Cohen, J.; Rankine, D.; Taylor, M.; Cambell, J.; Stephenson, T. Characterizing heat stress on livestock using the temperature humidity index (thi)—Prospects for a warmer caribbean. Reg. Environ. Change 2018, 18, 2329–2340. https://doi.org/10.1007/s10113-018-1359-x*
27. *Lewis Baida, B.E.; Swinbourne, A.M.; Barwick, J. et al. Technologies for the automated collection of heat stress data in sheep. Anim Biotelemetry 2021, 9, 4. https://doi.org/10.1186/s40317-020-00225-9*
28. *Marai, I.; Ayyat, M. & Abd El-Monem, U. Growth Performance and Reproductive Traits at First Parity of New Zealand White Female Rabbits as Affected by Heat Stress and Its Alleviation under Egyptian Conditions. Trop. Anim. Health Prod. 2001, 33, 451–462. https://doi.org/10.1023/A:1012772311177*
29. *Sellier N.; Guettier E.; Staub C. A review of methods to measure animal body temperature in precision farming. Am J Agric Sci Tech. 2014, 2:74–99. https://doi.org/10.7726/ajast.2014.1008.*
30. *Alhidary I.; Shini S.; Al Jassim R.; Gaughan J. Physiological responses of australian merino wethers exposed to high heat load. J Anim Sci. 2012, 90:212–20. https://doi.org/10.2527/jas.2011-3972.*
31. *Taylor N.A.; Tipton M.J.; Kenny G.P. Considerations for the measurement of core, skin and mean body temperatures. J Therm Biol. 2014, 46:72–101. https://doi.org/10.1016/j.jtherbio.2014.10.006.*
32. *Godyn D.; Herbut P.; Angrecka S. Measurements of peripheral and deep body temperature in cattle—a review. J Therm Biol. 2019, 79:42–9. https://doi.org/10.1016/j.jtherbio.2018.11.011.*
33. *Hyder I.; Ravi Kanth Reddy P.; Raju J.; Manjari P.; Srinivasa Prasad C.; Aswani Kumar K. et al. Alteration in rumen functions and diet digestibility during heat stress in sheep. In: Sejian V, Bhatta R, Gaughan J, Malik PK, Naqvi SMK, Lal R, eds. Sheep production adapting to climate change Singapore: Springer Nature; 2017. p. 235–65.*
34. *McManus, C.; Tanure, C.B.; Peripolli, V.; Seixas, L.; Fischer, V.; Gabbi, A.M.; Costa, J.B.G., Jr. Infrared thermography in animal production: An overview. Comput Electron. Agric. 2016, 123, 10–16. https://doi.org/10.1016/j.compag.2016.01.027*
35. *Čukić, A.; Rakonjac, S.; Djoković, R.; Cincović, M.; Bogosavljević-Bošković, S.; Petrović, M.; Savić, Ž.; Andjušić, L.; Andjelić, B. Influence of Heat Stress on Body Temperatures Measured by Infrared Thermography, Blood Metabolic Parameters and Its Correlation in Sheep. Metabolites 2023, 13, 957. https://doi.org/10.3390/metabo13080957*
36. *Lee Y.; Bok J.D.; Lee H.J.; Lee H.G.; Kim D.; Lee I. et al. Body temperature monitoring using subcutaneously implanted thermo-loggers from holstein steers. Asian-Australasian Association of Animal Production Societies; 2016. Report No.: 1011–2367 Contract No.: 2.*
37. *Torrao N.A.; Hetem R.S.; Meyer L.C.; Fick L.G. Assessment of the use of temperature-sensitive microchips to determine core body temperature in goats. Vet Rec. 2011, 168:328–34. https://doi.org/10.1136/vr.c6200*
38. *Kellogg D.L. In vivo mechanisms of cutaneous vasodilation and vasoconstriction in humans during thermoregulatory challenges. J Appl Physiol. 2006, 100:1709–18. https://doi.org/10.1152/japplphysiol.01071.2005*
39. *Silanikove N. The physiological basis of adaptation of goats to scarcity of food and water in harsh environments. Small Rumin. Res. 2000, 35:181-193. DOI: 10.1016/S0921-4488(99)00096-6*
40. *Khalifa H.H.; Shalaby T.; Abdel- Khalek T.M.M. An approach to develop a biometeorological thermal discomfort index for sheep and goats under Egyptian conditions. In: Proceeding of the 17th International Congress of Biometeorology (International Society of Biometeorology). Garmisch-Partenkirchen, Germany: Offenbach am Main; 2005. pp. 118-122*
41. *Nejad, J.G.; Sung, K.-I. Behavioral and Physiological Changes during Heat Stress in Corriedale Ewes Exposed to Water Deprivation. J. Anim. Sci. Technol. 2017, 59, 13 DOI 10.1186/s40781-017-0140-x*
42. *Shinde A.K.; Sejian V. Sheep husbandry under changing climate scenario in India: an overview. Indian J Anim Sci 2013, 83(10):998–1008*
43. *Chedid, M.; Jaber, L.S.; Giger-Reverdin, S.; Duvaux-Ponter, C.; Hamadeh, S.K. Water Stress in Sheep Raised under Arid Conditions. Can. J. Anim. Sci. 2014, 94, 243–257. https://doi.org/10.4141/cjas2013-188*

44. *Silanikove, N. The Physiological Basis of Adaptation in Goats to Harsh Environments. Small Rumin. Res. 2000, 35, 181–193. https://doi.org/10.1016/S0921-4488(99)00096-6*
45. *Cain, J.W., III; Krausman, P.R.; Rosenstock, S.S.; Turner, J.C. Mechanisms of Thermoregulation and Water Balance in Desert Ungulates. Wildl. Soc. Bull. 2006, 34, 570–581. https://doi.org/10.2193/0091-7648(2006)34(570:MOTAWB)2.0.CO;2*
46. *Gootwine, E. Mini Review: Breeding Awassi and Assaf Sheep for Diverse Management Conditions. Trop. Anim. Health Prod. 2011, 43, 1289–1296 https://doi.org/10.1007/s11250-011-9852-y*
47. *Moradi, M.H.; Nejati-javaremi, A.; Moradi-shahrbabak, M.; Dodds, K.G. Genomic Scan of Selective Sweeps in Thin and Fat Tail Sheep Breeds for Identifying of Candidate Regions Associated with Fat Deposition. BMC Genet. 2012, 13, 10. https://doi.org/10.1186/1471-2156-13-10*
48. *Shilja S.; Sejian V.; Bagath M.; Mech A.; David C.G.; Kurien E.K.; Varma G. and Bhatta R. Adaptive capability as indicated by behavioural and physiological responses, plasma HSP70 level, and PBMC HSP70 mRNA expression in Osmanabadi goats subjected to combined (heat and nutritional) stressors. International Journal of Biometeorology 2016, 60, 1311–1323.*
49. *Attia, N.E.-S. Physiological, Hematological and Biochemical Alterations in Heat Stressed Goats. Benha Vet. Med. J. 2016, 31, 56–62.*
50. *Chedid M.; Jaber L.S.; Giger-Reverdin S.; Duvaux-Ponter C. and Hamadeh S.K. Water stress in sheep raised under arid conditions. Can. J. Anim. Sci. 2014 94, 243–257.*
51. *Berihulay, H.; Abied, A.; He, X.; Jiang, L.; Ma, Y. Adaptation Mechanisms of Small Ruminants to Environmental Heat Stress. Animals 2019, 9, 75. https://doi.org/10.3390/ani9030075*
52. *Marai, I.F.M.; Daader, A.H.; Makkawy; M.Y., Gabr, H.A.; Ibrahim, H. Effect of housing system, season of year and age on some physiological parameters and blood constituents of Ossimi sheep. J. Arid Environ. 1992, 22, 277-285.*
53. *Salem, J.A.; Kobeisy, M.A.; Zenhom, M.; Hayder, M. Effect of season and ascorbic acid supplementation on some blood constituents of suckling Chois lambs and its crosses with Ossimi sheep in Upper Egypt. Assiut Journal of Agricultural Science 1998, 29, 87–100.*
54. *Srikandakumar, A.; Johnson, E.H.; Mahgoub, O. Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. Small Rumin. Res. 2003, 49 (2), 193–198. https://doi.org/10.1016/S09214488(03)00097-X*
55. *Aleena J.; Pragna P.; Archana P.R.; Sejian V.; Bagath M.; Krishnan G.; Manimaran A.; Beena V.; Kurien E.K.; Varma G. and Bhatta R. Significance of metabolic response in livestock for adapting to heat stress challenges. Asian J. Anim. Sci 2016, 10, 224–234.*
56. *Tadesse, D.; Patra, A.K.; Puchala, R.; Goetsch, A.L. Effects of High Heat Load Conditions on Blood Constituent Concentrations in Dorper, Katahdin, and St. Croix Sheep from Different Regions of the USA. Animals 2022, 12(17), 2273. https://doi.org/10.3390/ani12172273*
57. *III, J. W. C.; Krausman, P. R.; Rosenstock, S. S.; Turner, J. C. Mechanisms of thermoregulation and water balance in desert ungulates. Wildl. Soc. Bull. 2006, 34 (3), 570-581. https://doi.org/10.2193/0091-7648(2006)34(570:MOTAWB)2.0.CO;2*
58. *Abdul Niyas, P.A.; Chaidanya, K.; Shaji, S.; Sejian, V.; Bhatta, R.; Bagath, M.; Rao, G.S.L.H.V.P.; Kurien, E.K.; Girish, V. Adaptation of Livestock to Environmental Challenges. J. Vet. Sci. Med. Diagn. 2015, 4, 1–7. http://dx.doi.org/10.4172/2325-9590.1000146*
59. *Xu, S.; Luosang, J.; Hua, S.; He, J.; Ciren, A.; Wang, W.; Tong, X.; Liang, Y.; Wang, J.; Zheng, X. High Altitude Adaptation and Phylogenetic Analysis of Ti- Betan Horse Based on the Mitochondrial Genome. J. Genet. Genom. 2007, 34, 720–729. https://doi.org/10.1016/S1673-8527(07)60081-2*
60. *Kim, E.; Elbeltagy, A.R.; Aboul-naga, A.M.; Rischkowsky, B.; Sayre, B.; Mwacharo, J.M.; Rothschild, M.F. Multiple Genomic Signatures of Selection in Goats and Sheep Indigenous to a Hot Arid Environment. Heredity 2016, 116, 255–264. https://doi.org/10.1038/hdy.2015.94*
61. *Kosgey I.S.; Okeyo A.M. Genetic improvement of small ruminants in low-input, smallholder production systems: technical and infrastructural issues. Small Rumin Res 2007 70(1):76–88*
62. *Chauhan, S. S.; Celi, P.; Leury, B. J.; Clarke, I. J. & Dunshea, F. R. Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. Journal of Animal Science 2014 92(8), 3364-3374.*
63. *Friggens, N.C.; Blanc, F.; Berry, D.P.; Puillet, L. Review: Deciphering animal robustness. A synthesis to facilitate its use in livestock breeding and management. Animal 2017, 11, 2237–225.*
64. *Gaughan, J.B.; Sejian, V.; Mader, T.L.; Dunshea, F.R. Adaptation strategies: ruminants. Anim. Front. 2019, 9, 47–53.*
65. *Osei-Amponsah, R.; Chauhan, S.S.; Leury, B.J.; Cheng, L.; Cullen, B.; Clarke, I.J.; Dunshea, F.R. Genetic Selection for Thermotolerance in Ruminants. Animals 2019, 9, 948. https://doi.org/10.3390/ani9110948*

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VARIABILITY OF MORPHOLOGICAL AND GROWTH CHARACTERISTICS OF THE WURTTEMBERG SHEEP BREED

VARIJABILNOST MORFOLOŠKIH I OSOBINA PORASTA VIRTEMBERG RASE OVACA

Aleksandar Čukić [a*], Božidar Milošević[a], Željko Savić[a] and Ljiljana Andjušić[a]

[a] Faculty of Agriculture, University of Priština in Kosovska Mitrovica, Kopaonička bb, 38219 Lešak, Serbia
*Corresponding author: aleksandar.cukic@pr.ac.rs

A.Č. https://orcid.org/0000-0001-9063-3431, B.M. https://orcid.org/0000-0003-4381-0276, Ž.S. https://orcid.org/0009-0006-5932-3551, Lj.A. https://orcid.org/0000-0002-0054-1128

ABSTRACT

The aim of this study was to determine and present the basic characteristics of Wurttemberg sheep, which includes analyzes of the characteristics of body measurements of sheep, with detailed statistical analysis, in order to define the degree of variability of characteristics, as well as to investigate environmental influences (sex, year birth, season and type of birth) on the examined traits using general linear method and estimating genetic parameters for lamb growth traits, using BreedR software package in R statistical programming environment. The research was conducted on a sample of 964 sheep, 860 females and 104 males. The following morphological characteristics were examined: Height to withers, body length, girth of chest, depth of chest, witdh of chest, width of pelvic and circumference of the testicles of rams. From the physiological traits, i.e. production traits, the following were examined: body weight at birth, body weight at 30 days and body weight at 90 days of age, i.e. weight at weaning, weight in the first year and weight at 3 years. The results of the research showed that the characteristics of the body measurements of sheep and rams, are similar or equal to the characteristics that are standard for the breed, while in sheep the height to withers and the girth of chest were at lower values than the standard. Body weight values in the first as well as at the age of three were at the level of breed standards, while the weight of lambs at birth was lower than the standard. Sex and year of birth had a statistically highly significant (** = P <0.001) influence on all examined morphological traits, while in addition to these factors, body lenght was influenced by the type of birth and width of chest by birth season. The year and type of birth had a statistically highly significant (** = P <0.001) influence on the growth characteristics of lambs, while the sex and year of birth had influence on body weight in the first year as well as at the age of 3 years. Heritability was at the level of medium values, for the trait of body weight at birth 0.30, for body weight at 30 days 0.23, the highest for body weight at weaning 0.38.

Keywords: Wurttemberg sheep, body measurements, growth traits, environmental factors, heritability.

SAŽETAK

Cilj ove studije bio je da se utvrde i predstave osnovne karakteristike virtemberške ovce, pri čemu su u istraživanje uključene analize osobina telesnih mera ovaca, uz detaljnu statističku analizu, u cilju definisanja stepena varijabilnosti osobina, kao i da se istraže uticaji okoline (pol, godina rođenja, sezona i tip rođenja) na ispitivane osobine primenom opšte linearne metode i procenom genetskih parametara za osobine porasta jagnjadi, korišćenjem softverskog paketa BreedR u okruženju za statističko programiranje R. Istraživanje je sprovedeno na uzorku od 964 grla, 860 ženki i 104 mužjaka. Ispitivane su sledeće morfološke karakteristike: visina grebena, dužina tela, obim grudnog koša, dubina grudnog koša, širina grudnog koša, širina karlice i obim testisa ovnova. Od fizioloških, odnosno proizvodnih osobina, ispitivane su: telesna masa pri rođenju, telesna masa sa 30 dana i telesna masa u dobi od 90 dana, odnosno težina pri odbijanju, težina u prvoj godini

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i težina sa 3 godine. Rezultati istraživanja su pokazali da su karakteristike telesnih mera ovaca i ovnova slične ili jednake karakteristikama koje su standardne za rasu, dok su kod ovaca visina grebena i obim grudnog koša bili na nižim vrednostima od standardne. Vrednosti telesne mase u prvoj kao i u uzrastu od tri godine bile su na nivou standarda rase, dok je težina jagnjadi pri rođenju bila niža od standarda. Pol i godina rođenja imali su statistički visoko značajan (** = P <0.001) uticaj na sve ispitivane morfološke osobine, dok su pored ovih faktora na dužinu tela uticali tip rođenja i sezona na širinu grudnog koša. Godina i tip rođenja imali su statistički visoko značajan (** = P <0.001) uticaj na osobine porasta jagnjadi, dok su pol i godina rođenja imali uticaj na telesnu masu u prvoj godini, kao i u uzrastu od 3 godine. Heritabilnost je bila na nivou srednjih vrednosti, za osobinu telesne mase pri rođenju 0.30, za telesnu masu sa 30 dana 0.23, najviša za telesnu masu pri odbijanju 0.38.

Ključne reči: Virtemberg ovca, telesne mere, proizvodne osobine, faktori okoline, heritabilnost.

1. INTRODUCTION

The Wurttemberg sheep (Merino Landschaf) is a typical breed for meat and wool production that is widespread, and in the area of the Balkan it is used for crossing with Pramenka sheep in order to increase fattening characteristics and meat yield. A serious undertaking to improve the production of lamb meat was made by introducing the Wurttemberg sheep into sheep breeding programs in Serbia (1). Many scientific studies have confirmed the opinion that this breed is used only in the programs of industrial crossbreeding of some local populations in order to increase the growth of lambs. Due to the existence of meat breeds, with more pronounced fattening characteristics, and above all higher quality of lamb carcasses and better physical, chemical and sensory properties of meat, breeding Wurttemberg sheep in pure breed is not of interest (2, 3, 4, 5, 6, 7).

According to the data of the selection service, and recent research, the body weight of adult sheep ranges from 70-75 kg, and rams 100-120 kg, the height to withers in sheep averages about 70 cm, and rams 80 cm, the body weight of lambs at birth is about 4.5 kg. In conditions of intensive fattening, lambs reach a body weight of 25-30 kg at the age of 90 days. The meat yield ranges from 52-56%. The yield of wool per sheep is 4-4.5 kg, and in rams 5-6 kg. The wool yield is about 50%. Milk production is weak or moderate, although milk is mainly used for raising offspring. It starts with mating at 10 (female) or 12 months of age (male). Out of 100 sheep, an average of 120-140 lambs are obtained. Due to the excellent body structure, good production, good health and very good acclimatization ability, this breed has been exported, and even today it is exported to many countries around the world. This breed was especially important for the improvement of sheep production in our country because, above all, it participated in the creation of Pirot's breed sheep. In addition, in recent years she has been the main ameliorator of many other strains of Pramenka in our country, with very good results (8). In the coming period, Serbian sheep breeding should work on improving the genetic potential of sheep, and this can be achieved by crossing domestic breeds with rams of the Wurttemberg breed, creating new genotypes for meat production and creating new genotypes for combined production (7).

Linear body measurements on live animals are widespread and are used in research work as a simple tool for recording certain aspects of animal growth and shape (9, 10). Linear measurements can be used to assess growth rate, weight, food utilization, and carcass characteristics, to monitor the relationship between production performance, visual assessment, and physical measurements (11, 12). The production characteristics of sheep depend on a number of genetic and non-genetic factors, in order to successfully organize sheep production we must have a breeding program, and know which factors affect production (13). Among them are year and season, which is primarily reflected through nutrition, housing and care of animals during the production cycle, especially during pregnancy (14).

In order for the selection to be successfully carried out for the purpose of obtaining higher sheep meat production, it is necessary to know the external factors on which the quantity and quality of meat depend (accommodation, nutrition, care), as well as genetic parameters (heritability, repitability, genetic

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correlations) that have an economic effect on production (15). With known high values of heritability coefficients, it is possible, based on phenotypic expressiveness, to predict the genetic value of an individual, which is considered important in selection programs (16).

There is a relatively small number of such studies in this breed, so the main goal of this study is to look at some of the indicators of variability of morphological and physiological characteristics of sheep, comparing the animals of different farms, but also comparing the variability of examined traits, phenotypes, under the influence of different factors and examine the heritability coefficient for lamb growth traits.

2. MATERIALS AND METHODS

The data used in this research were collected over a period of 8 years, taken from the database of the basic breeding organization "Association of Farmers" Batocina, which includes sheep raised in the Sumadija district. Based on the examined 964 sheep, there were 860 females and 104 males. Data entered into the sheep registry program, which is constructed in the form of a relational database, were extracted and classified using SQL (Structured Query Language) codes, and then the elimination of incorrectly entered, extreme values of individual parameters was performed using program codes in R statistical software environment, in which were calculated the basic parameters of descriptive statistical analysis, environmental impact on the examined traits using a general linear model, as well as further processing using the method of best linear indicators BLUP to assess the genetic value of animals.

Examination of phenotypic expression and variability of traits was performed by calculating the basic parameters of descriptive statistical analysis such as: arithmetic mean, standard error of arithmetic mean, standard deviation (SD), coefficient of variation (CV (%)), interval of variation (min - max).

From the morphological traits the following were examined: Height to Withers (HW), Body Length (BL), Girth of Chest (GC), Depth of Chest (DC), Witdh of Chest (WC), Width of Pelvic (WP) and circumference of the testicles of rams (CT).

From the physiological traits, i.e. production traits, the following were examined: body weight at birth (BW0), body weight at 30 days (BW30) and body weight at 90 days (BW90=WW) of age, i.e. weight at weaning (weaning weight), weight in the first year (BW1) and weight at 3 years (BW3).

Statistical analysis of the influence of a number of non-genetic factors on the manifestation of morphological and production characteristics of growth was examined. The analysis of the influence of non-genetic environmental factors was performed using a general linear model. The application of this procedure enables the simultaneous analysis of several different influences, regardless of whether they are categorical or continuous factors by their nature. For estimating the effects and testing hypotheses, the general linear model is based on the application of least squares methods. Data were also processed in a free licensed programming environment (17), using the program Least-Squares Means.

By analyzing the influence of non-genetic environmental factors using a general linear model, the influences of the following factors were examined: Influence of sex, influence of year of birth, birth season and influence of birth type.

The following model was used to analyze the influence of individual non-genetic factors on body measurements traits:

$$y_{ijkmn} = \mu + G_r + P_l + R_s + T_r + e_{ij\kappa lmn},$$

in which:

$\mathbf{y_{ijkmn}}$ - individual of the i-th farm, j-th group of the year of birth, k-th sex, m-th season of birth, fixed influence of the n-th type of birth.

μ - general average of the population with equal representation of all classes of influence (Oi + GLj + STk + Srl + Grm + GrSrml),

Gr - fixed influence of the j-th year of birth,

Pl - fixed influence of the k-th sex,

Rs - fixed influence of the m-th season of birth,

Tr - fixed influence of the n-th type of birth and

$\mathbf{e_{ijklmn}}$ - other indeterminate influences.

The calculation of genetic parameters was performed using the software package BreedR (18), in the R statistical software environment, which uses the program BLUPF90 (19) using the REML (average information restricted maximum likelihood) method. An animal model was used to

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define additive genetic and environmental variance. The software solutions used in the research are open source, i.e. free.

Heritability (h^2) was singled out for traits: body weight at birth (BW0), body weight at 30 days (BW30) and body weight at 90 days (BW90=WW) of age, i.e. weight at weaning (weaning weight).

3. RESULTS AND DISCUSSION

3.1. DESCRIPTIVE STATISTICS FOR BODY MEASUREMENTS TRAITS

The following tables show the results of examining the characteristics of the body measurements of the Wurttemberg breed, which were bred in the Sumadija district. Based on the collected data, the basic parameters of descriptive statistics for these traits were determined, and the influence of a number of non-genetic factors on the manifestation of these traits was presented using a general linear model. Table 1. shows the results of descriptive statistics for the morphological characteristics of sheep and rams, while the table 2. shows the results of descriptive statistics for the physiological characteristics.

Table 1. Morphological characteristics of sheep and rams

	Sex	HW	WC	BL	DC	WP/CT	GC
n	male	104	104	104	104	104	104
	female	860	860	860	860	860	860
$\bar{X}$	male	80.3	37	84.1	39.4	31.8	123
	female	66.2	31.7	73.5	35.9	27	91.8
$S_{\bar{x}}$	male	0.20	0.23	0.399	0.27	0.596	1.250
	female	0.11	0.07	0.132	0.05	0.039	0.147
SD	male	2.05	2.33	4.07	2.74	6.08	12.8
	female	3.17	1.95	3.88	1.41	1.14	4.31
min	male	75	33	74	34	23	93
	female	56	24	57	30	20	80
max	male	87	44	95	46	41	150
	female	79	37	91	40	30	123

The average height to withers in rams was 80.3 cm, which is approximately the same value of the HW as pointed out by many authors, 80 cm on average (1, 20), as well as in accordance with the Institute of Animal Husbandry (8). Chest width averaged 37 cm, body length averaged 84.1 cm, in the range of 74-95 cm. The average chest depth of rams was 39.4 cm, ranging from 34-46 cm. Testicular circumference was 31.8 cm, 23 cm minimum and 41 cm maximum. The average chest girth of rams was 123 cm, in the range of 93-150 cm.

The average height to withers in sheep was 66.2 cm, which is a lower value compared to the HW pointed out by many authors, 70 cm on average (1, 20, 21). In the results of the research obtained by Petrović et al. (21), body lenght in sheep is smaller, on average 72.11 cm, pelvic width is also smaller, on average 25.82 cm, while in our results the body length is 73.5 cm, pelvic width 27 cm, while chest girth is larger, on average 97.66 cm, in our results it is 91.88 cm.

Table 2. Physiological characteristics of sheep and rams

	Sex	BW0	BW30	BW90	BW1	BW3
n	male	104	104	104	104	104
	female	860	860	860	860	860
$\bar{X}$	male	3.71	15.4	27.6	85.8	94.1
	female	3.59	14.7	27	62.5	67.7
$S_{\bar{x}}$	male	0.068	0.137	0.249	0.653	0.793
	female	0.018	0.047	0.084	0.142	0.118
SD	male	0.692	1.39	2.54	6.66	8.09
	female	0.516	1.39	2.48	4.17	3.45
min	male	2.6	12	23	70	83
	female	2.3	10	22	52	57
max	male	5.5	19	34	120	132
	female	5.6	26	38	92	98

The average birth weight was 3.71 kg in male lambs and 3.59 kg in females, Petrović (1)

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found a higher value for this trait, which was 4.95 kg. Also, Skalicki and Ristevski (22) found a higher value for the body weight of lambs at birth in the Wurttemberg breed imported to Macedonia, where the value was 4.05 kg. According to Krogmeier (23), the body weight of Wurttemberg lambs at birth in Germany is 4.90 kg. The body weight of male lambs on weaning was on average 27.6 kg, and female 27 kg, which is approximately with the research of Petrovic (1) who states that lambs reach a weight of 25-30 kg on average with 90 days.

Mekic et al. (20) stated that the body weight of one-year-old animals is 50-55 kg in females and 90-110 kg in males, 110-130 kg in adult rams, and 60-75 kg in sheep. In our research, the body weight in the first year in males averaged 85.8 kg, in the range of 70-120 kg, in females 62.5 kg, ranged from 52-92 kg. Body weight at 3 years in rams averaged 94.1 kg, in intervals of 83-132 kg, in sheep an average of 67.7 kg, from a minimum of 57 kg to a maximum of 98 kg, which is within the breed standard.

3.2. ANALYSIS OF THE INFLUENCE OF ENVIRONMENTAL FACTORS ON EXAMINED TRAITS

Tables 3 and 4 show the analysis of the influence of environmental factors on examined traits, in table 3 on morphological and in table 4 on physiological traits.

Table 3. Results of the linear model of analysis of morphological characteristics.

Traits	Factors			
	Sex	Year of birth	Birth season	Birth type
HW	< .001	< .001	0.019	0.180
BL	< .001	< .001	0.021	< .001
DC	< .001	< .001	0.020	0.005
WC	< .001	< .001	< .001	0.100
GC	< .001	< .001	0.119	0.649
WP/CT	< .001	< .001	0.174	0.923

ns=P>0,005; *=P<0,005; **=P<0,001;

The analysis of the results of the morphological traits of sheep and rams, using a general linear model, found a statistically highly significant (** p <0.001) influence of sex and year of birth on all traits, while in addition to these factors, body lenght was influenced by birth type, and width of chest by birth season. It was determined that the effect of sex was significant for all traits of body measurements, where males had higher values for all traits compared to females. Similar results in their research are confirmed by Petrović et al. (21) also in the Wurttemberg breed, Jafari and Hashemi (24) in the Makui, Lalit et al. (25) in Harnali breed and Dauda et al. (26) in Balami breed. Dauda et al. (26) stated that the superiority of males over females in some physical traits may be the result of the secretion of the hormone testosterone secreted by males. As in these studies, so did Kumar et al. (27), as well as Das et al. (28) in their research stated that the type of birth and the season of birth had an impact on certain characteristics of body measurements, while on most did not.

Table 4. Results of the linear model of analysis of physiological characteristics.

Traits	Factors			
	Sex	Year of birth	Birth season	Birth type
BW0	0.037	< .001	0.776	< .001
BW30	0.008	< .001	0.014	< .001
BW90	0.002	< .001	0.010	< .001
BW1	< .001	< .001	0.044	0.151
BW3	< .001	< .001	0.840	0.642

ns=P>0,005; *=P<0,005; **=P<0,001;

According to the results of the analysis of the general linear model, a statistically highly significant (**p<0.001) influence of year and type of birth on BW0, BW30 and BW90 was determined. Many authors (14, 29, 30) conclude that the year and type of birth have an impact on the characteristics of the body mass of lambs growing and at birth, while in the research of Zeljić et al. (31) in Bergamo lambs, Boujenane and Dialla (32) in Sardi lambs, year and type of birth had an impact on body weight at birth, while on body weight at 30 days and on weaning did not. Body weight at birth and weaning depends on many environmental factors. Among them are the year and the season, which is primarily reflected in the nutrition, housing

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and care of animals during the production cycle, especially during pregnancy (14).

Sex and year of birth had the statistically highly significant (**p<0.001) influence on body weight in the first year, as well as at the age of 3. As it is generally known that the increase in males is higher, so it has been proven in this study, and the same is confirmed by many authors (30, 33, 34, 35, 36). The year of birth had an impact on these traits, which has also been confirmed in many studies (34, 37, 38, 39), and Rahimi et al. (39) in their study of Makui sheep stated that this could be due to differences in farm management, food availability, disease, and climate (rainfall, humidity, and temperature) that affect pasture quality and quantity in different years.

3.3. HERITABILITY COEFFICIENT FOR LAMB GROWTH TRAITS

The results of the assessment of genetic parameters for growth characteristics of lambs, i.e. the heritability coefficient for these characteristics, are shown in table 5.

Table 5. Values of heritability (h^2) and standard errors of heritability (S_h^2) for growth characteristics of lambs.

Traits	h^2	S_h^2
BW0	0.3022	0.0973
BW30	0.2392	0.1032
BW90	0.3828	0.1086

low heritability = < 0.2; medium heritability = 0.2-0.5; high heritability = >0.5

Heritability was at the level of medium values, for the trait of body weight at birth 0.3022, for body weight at 30 days 0.2392, highest for body weight at weaning 0.3828. The value of heritability for body weight at birth is higher in our research compared to many, where it ranged from 0.03 (40) in the Romney Marsh lambs to 0.46 (41) in the Menz lambs. For body weight at 30 days and at weaning is more or less in relation to many studies, where it has been proven that the coefficient of heritability for these traits in lambs ranges from low to medium values (15, 36, 40, 41, 42, 43, 44, 45, 46).

If we compare our research with the research of Zeljić et al. (15) in Sjenicka Pramenka, the coefficient of heritability is at significantly higher values for body weight at birth, where it was 0.03, lower for body weight at 30 days, 0.46 in their results, and higher for body weight at weaning in relation to their results where it was 0.30. Determined coefficient values and standard error of heritability differ considerably between some authors what can be a consequence of research conducted in different populations, breeds, types of sheep (different size and structure of samples), different conditions of keeping as well as various applied models for calculating heritability. In addition, migration, selection and inbreeding can change the value of this coefficient (15).

4. CONCLUSIONS

Based on the given results for sheep and rams of the Wurttemberg breed examined in this study, most of the examined parameters were found to meet breed standards, while some traits show a lower value compared to the breed standards and traits researched and ascertained by domestic authors for this breed of sheep. Results of this study showed that certain non-genetic factors have a significant influence on the morphological and growth characteristics of the Wurttemberg sheep breed. Sex and year of birth had a statistically highly significant influence on all morphological traits, while in addition to these factors, body lenght was influenced by the type of birth and width of chest by birth season. The year and type of birth had the highest influence on the growth characteristics of lambs, while on the body weight in the first and in the third year, a year of birth and sex. The coefficient of heritability was at the level of medium values, for body weight at birth much higher in our research compared to many, while for body weight at 30 days and at weaning more or less in relation to many studies. All examined characteristics are influenced by diet, care, manner of keeping and selection, which means that we must constantly work on improving all these conditions as well as on more correct selection. This breed is constantly being tested with the aim of obtaining the highest quality breeding material, so it is necessary to do more such research, in order to contribute to improving the selection of this breed.

REFERENCES

1. *Petrović P.M. Examination of reproductive and production traits of the Württemberg sheep breed (Merino Landschaf). Doctoral dissertation, Faculty of Agriculture, Belgrade, 1992.*
2. *Živković R., Mitić N., Kostić V. Virtemberška ovca i mogućnost gajenja u uslovima Homolja, Zbornik radova institut za stočarstvo, Beograd, pp. 110-118, 1969.*
3. *Čaušević Z. Prilog poznavanju uticaja aklimatizacije na neka vunska svojstva finorunih ovaca. (izvod iz disertacije). Zbornik radova za poljoprivredna istraživanja, Sarajevo, 1972.*
4. *Mitić N., Skalicki Z., Djordjevic V., Manić M. Properties of crosses created by a combination of crossbreeding with 3 breeds of sheep. Arhiv za poljoprivredne nauke 1982, 43, 279-297.*
5. *Petrović P.M. Genetic and improvement of sheep. Scientific Book, Belgrade, 2000, 365 pp.*
6. *Mekić C., Krajinović M., Žujović M., Trifunović G., Perišić P. Reproduktivni pokazatelji oplemenjene pramenke sa virtemberškom rasom u zavisnosti od uslova odgajivanja. Savremena poljoprivreda 2003, 52, 3–4, 225–228.*
7. *Jevtić A. Influence of genotype and condition in different breeding systems on reproductive and production characteristics of sheep. Doctoral dissertation, University of Prishtina, 2007.*
8. *Main breeding program in animal husbandry. Statistical Yearbook, Institute of Animal Husbandry, Belgrade, 2019.*
9. *Salako A.E. Application of morphological indices in the assessment of type and Function in sheep. International Journal of Morphology 2006, 24, 13 – 8.*
10. *Alphonsus C., Finangwai H.I., Yashim S.M., Agubosi O.C.P., Sam I.M. Effect of dam parity on measures of growth in Red sokoto goats 1, 3, 6, and month of age. Continental J. Animal and Veterinary Research 2010. 2, 9-13.*
11. *Lawrence T.I.J., Fowler V.R. Growth on farm animals. CAB international pp. 271– 283, 1998.*
12. *Fourie P.J, Neser F.W.C., Olivier J.J., Der Westhuizen C.V. Relationship between production performance, visual appraisal and body measurements of young Dorper rams. S. Afr. J. Anim. Sci. 2002, 32,256-262.*
13. *Ugarte E.nThe breeding program of Latxa breed. Biotechnology in Animal Husbandry 2007, 23, 97-111.*
14. *Petrović M.P., Caro Petrović V., Muslić R.D., Maksimović N., Petrović M.M., Ilić Z., Stojković J. Effect of genetic and environmental factors on the phenotype characteristics of lambs. Biotechnology in Animal Husbandry 2015, 31 (2), 223-233.*
15. *Zeljić K., Stanojević D., Bogdanović D., Bogdanović V., Mekić C., Ružić-Muslić D., Maksimović N., Stojiljković N. Estimation of growth traits heritability coefficients in sheep. Biotechnology in Animal Husbandry 2019, 35 (4), 347-355.*
16. *Visscher P.M., Hill W.G., Wray N.R. Heritability in the genomics era- concepts and misconceptions. Nature Reviews Genetics 2008, 9(4), 255-266*
17. *R Core Team 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.*
18. *Muñoz F., Sanchez L. BreedR: Statistical Methods for Forest Genetic Resources Analysts. R package version 0.12-2. 2018. https://github.com/famuvie/breedR.*
19. *Misztal I.S., Tsuruta D.A., Lourenco L., Masuda Y., Aguilar I., Legarra A., Vitezica Z. Manual for BLUPF90 family programs. University of Georgia, 2018.*
20. *Mekić C., Latinović D., Grubić G. Odgajivanje, reprodukcija, selekcija i ishrana ovaca, Univerzitet u Beogradu, Poljoprivredni fakultet, 2007, str. 142-145.*
21. *Petrović M.P., Petrović V.C., Muslić R.D., Ilić Z., Spasić Z., Stojković J., Maksimović N. Genetic and phenotypic characteristics of the body measured traits in Merinolandschaf breed of sheep. Biotechnology in Animal Husbandry 2012, 28 (4): 733-741.*
22. *Skalicki Z. and Ristevski T. Produktivnost virtemberške rase ovaca. Savremena poljoprivreda 1998, 48, (1–2), 91–96.*
23. *Krogmeier D., Wenzlaff O., Dzapo V., Wassmuth R. Heterotic effects in sheep. Correlations between the viability of newborn lambs and selected metabolic parameters - results of a reciprocal crossbreeding trial. Züchtungskunde 1990, 62, 1, 52-65.*
24. *Jafari S., Hashemi A. Estimation of genetic parameters for body measurements and their association with yearling liveweight in the Makuie sheep breed. S. Afr. J. Anim. Sci. 2014, 44 (2): 141–147.*
25. *Malik Z.S., Dalal D.S., Patil C.S., Dahiya S.P. Genetic studies on growth, reproduction and wool production traits of Harnali sheep. Indian J. Anim. Res. 2016,51, 5, 813-816.*
26. *Dauda A., Okon B., Ibom L.A., Abbaya H. Y. Phenotypic Characterization of Balami Breed of Sheep in Maiduguri, North-Eastern Nigeria. Nigerian J. Anim. Sci. 2018, 20 (2): 26-32.*

27. *Kumar S., Dahiya S. P., Malik Z.S., Patil C.S. Prediction of body weight from linear body measurements in sheep. Indian J. Anim. Sci.2017, B-3360, 1-4.*
28. *Das A.K., Chakraborty, Dibyendu, Kumar, Nishant, Gupta, Parul, Khan, Nusrat N., Bukhari S. Efects of non-genetic factors on performance traits of kashmir merino sheep. Indian Journal of Animal Research. 2014, 48, 2, 106-108.*
29. *Ilić Z., Jevtić-Vukmirović A., Petrović M.P., Caro-Petrović V., Milošević B., Spasić Z., Ristanović B. Effect of mating method, sex and birth type on growth of lambs. Biotechnology in Animal Husbandry 2013, 29 (2), 277-286.*
30. *Petrović M.P., Ružić- Muslić D., Maksimović N., Memiši N. Effect of environmental and paragenetic factors on birth mass variability of MIS sheep populations. Biotechnology in Animal Husbandry 2009, 25 (3-4), 213-219.*
31. *Zeljić K., Stanojević D., Bogdanović J., Gligović N. Uticaj godine, pola i tipa rođenja na telesnu masu i porast jagnjadi bergamo rase ovaca. „XXVI savetovanje o biotehnologiji", Zbornik radova institut za stočarstvo, Beograd, 2021.*
32. *Boujenane I., Diallo I.T. Estimates of genetic parameters and genetic trends for pre-weaning growth traits in Sardi sheep. Small Ruminant Research 2016, 146: 61-68.*
33. *Dixit SP., Dhilon JS., Sing G. Genetic and non-genetic parameter estimates for growth traits of Bharat Merino lambs. Small Rumin Res 2001, 42, 101-104.*
34. *Matika O., Van Wyk JB., Erasmus GJ, Baker RL. Genetic parameter estimates in Sabi sheep. Livest Prod Sci 2003, 79, 17-28.*
35. *Abegaz S., Van Wyk J.B., Olivier J.J. Model comparisons and genetic and environmental parameter estimates of growth and the Kleiber ratio in Horro sheep, S. Afr. J. Anim. Sci. 2005, 35, 30-40.*
36. *Rashidi A., Mokharti M.S., Safari Jahanshahi A., Mohamadi Abdi M.R. Genetic parameter estimates of pre-weaning growth traits in Kermani sheep. Small Ruminant Research 2008, 74(1-3), 165-171.*
37. *Ozcan M., Ekiz B., Yilmaz A., Ceyhan A. Genetic parameter estimates for lamb growth traits and greasy fleece weight at first shearing in Turkish Merino sheep. Small Rumin Res 2005, 56,215-222.*
38. *Komlosi I. Genetic parameters for growth traits of the Hungarian merino and meat sheep breeds in Hungary. Appl Ecol Env Res 2008, 6, 77-84.*
39. *Rahimi S.M., Rafat S.A., Jafari S.S. Effects of environmental factors on growth traits in makuie sheep. Biotechnology in Animal Husbandry 2014, 30 (2), 185-192.*
40. *Perez J.O., Aldana T., Torres D., Grajales H., Manrique C. Estimación de parámetros genéticos de cuatro tipos raciales ovinos del trópico colombiano. Universidad Nacional de Colombia, Facultad de Medicina Veterinaria y Zootecnia, Departamento de Producción Animal Bogotá, Colombia, 2017.*
41. *Gizaw S., Lemma S., Komen H., Van Arendonk J.A.M. Estimates of genetic parameters and genetic trends for live weight and fleece traits in Menz sheep. Small Rumin. Res. 2007, 70, 145-153.*
42. *Yazdi M.H., Engstrom G., Nasholm A., Johansson K., Jorjani H., Liljedahl L.E. Genetic parameters for lamb weight at different ages and wool production in Ba-luchi sheep. Animal Science 1997, 65, 247–255.*
43. *Safari E., Fogarty N.M., Gilmour A.R. A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep. Livestock Production Science 2005, 92, 271-289.*
44. *Gamasee V.A., Hafesian S.H., Ahmadi A., Baneh H., Farhadi A., Mohamadi A. Estimation of genetic parameters for body weight at different ages in Mehraban sheep. African Journal of Biotechnology 2010, 9, 5218-5223.*
45. *Jafaroghli Rashidi A., Mokhtari M.S., Mirzamohammadi E. Estimation of genetic parameters for body weight traits in Baluchi sheep. Journal of Livestock Science and Technologies 2013, 1(2), 28-33.*
46. *Shahdadi A.R., Saghi D.A. Estimating Genetic Parameters of body weight traits in Kourdi sheep. Iranian Journal of Applied Animal Science 2016, 6(3), 657-663.*

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VARIJABILNOST LABORATORIJSKIH PARAMETARA METABOLIČKOG PROFILA U KETOZI KOD KRAVA – DA LI JE DOVOLJNO SAMO POREĐENJE SA REFERENTNIM VREDNOSTIMA?

VARIABILITY OF LABORATORY PARAMETERS OF THE METABOLIC PROFILE IN KETOSIS IN COWS - IS ONLY COMPARISON WITH REFERENCE VALUES SUFFICIENT?

Biljana Delić Vujanović[a*], Maja Došenović Marinković[a], Slaviša Đokić[b], Radojica Djoković[c], Miloš Ži. Petrović[c], Mira Majkić[d]

[a]Academy of applied studies Šabac, Dobropoljska 5, 15000 Šabac, Serbia;
[b] Veterinary clinic Djokic, Derventa, R.Srpska, BiH
[c]Faculty of Agronomy, Čačak, Serbija
[d]University of Novi Sad, Faculty of Agriculture, Department of veterinary medicine, Novi Sad, Serbia
*****Correspondence: biljana_delic@hotmail.com

M.D.M. https://orcid.org/0009-0007-8904-0197, R.Dj. https://orcid.org/0000-0003-0900-3227, M.Ž.P. https://orcid.org/0000-0002-4707-6796, M.M. https://orcid.org/0009-0007-0110-7327

SAŠETAK

Ketoza predstavlja značajni metabolički poremećaj kod krava koji nastaje kao posledica negativnog energetskog bilansa, kada raste koncentracija ketonskih tela u krvi, mleku i urinu. Ketoza krava je metabolička bolest koju se odlikuje poremećajem metabolizma ugljenih hidrata i masti sa povećanom proizvodnjom ketonskih tela u organizmu. Osnovne metaboličke adaptacije kod krava u ketozi su: povišena koncentracija BHB, povišena koncentracija NEFA, niža koncentracija glukoze, povišena vrednost jetrinih enzima i bilirubina, poremećaj makro i mirko elemenata, povišene vrednosti inflamatornih markera, oksidativni stres, insulinska rezistencija, a prate je i različite pridružene bolesti i poremećaji ponašanja. Obzirom da se ketoza često javlja u različitm formama, a da dobijene vrednosti metaboličkog profila kod većine parametara ostaju u okviru referentnihvrednosti, potrebno je ispitati varijabilnost krvnih parametara kako bi se preporučio novi način intepretacije metaboličkog profila u proceni ketoze kod krava. Očekujemo da ćemo utvrditi koji parametri pokazuju najveći stepen varijabilnosti, čime ćemo moći da damo praktični doprinos dijagnostici ketoze, jer ćemo utvrditi parametre koji su najsenzitivniji na ketozu, a čija varijabilnost u kombinaciji sa specifičnim promenama u vrednosti BHB omogućuju ne samo dijagnostiku ketoze već i graduisanje njene težine kod svake krave posebno. Svakodnevni praktični način procene zdravlja životinja ogleda se u poređenju dobijenih vrednosti sa referentnim vrednostima zbog toga je bilo potrebno da odredimo u kom procentu postoji veliko odstupanje vrednosti parametara (Z skor preko ili ispod 2), a koje u praktičnom smislu znači da je došlo do promene dobijenih vrednosti izvan referentnih. Kada se isključi BHB čije visoko odstupanje je u osnovi definicije ketoze i posmatraju se vrdnosti ostalih metaboličkih parametar, zaključujemo da u 360 merenja postoji 105 odstupanja od referentnih vrednosti, što je 29,17%, dok su u 70,83% merenja vrednosti metaboličkih parametara bili u okviru referentnih vrednosti. Navedeni rezultati ukazuju da se u praksi mora pristupiti novom načinu čitanja i interpretacije rezultata koje u obzir mora uzeti poziciju vrednostimetaboličkog parametra u okviru referentne vrednosti, a ne samo činjenicu da li se metabolički parametar nalazi izvan referentnih vrednosti.

Ključne reči: krave, ketoza, metabolički profil, z-skor.

ABSTRACT

Ketosis is a significant metabolic disorder in cows that occurs as a result of a negative energy balance, when the concentration of ketone bodies in the blood, milk and urine increases. Cow ketosis is a metabolic disease

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characterized by a disturbance in carbohydrate and fat metabolism with increased production of ketone bodies in the body. Basic metabolic adaptations in cows in ketosis are: increased concentration of BHB, increased concentration of NEFA, lower concentration of glucose, increased value of liver enzymes and bilirubin, disorder of macro and micro elements, increased values of inflammatory markers, oxidative stress, insulin resistance, and are accompanied by various associated diseases and behavioral disorders. Given that ketosis often occurs in different forms, and that the obtained values of the metabolic profile for most parameters remain within the reference values, it is necessary to examine the variability of blood parameters in order to recommend a new way of interpreting the metabolic profile in the assessment of ketosis in cows. We expect to determine which parameters show the greatest degree of variability, which will allow us to make a practical contribution to the diagnosis of ketosis, because we will determine the parameters that are most sensitive to ketosis, and whose variability in combination with specific changes in the BHB value allow not only the diagnosis of ketosis, but also the graduation its weight in each cow separately. The everyday practical way of assessing the health of animals is reflected in the comparison of the obtained values with the reference values, therefore it was necessary to determine in what percentage there is a large deviation of the parameter values (Z score above or below 2), which in a practical sense means that there has been a change obtained values outside the reference ones. When BHB is excluded, whose high deviation is the basis of the definition of ketosis, and the values of other metabolic parameters are observed, we conclude that in 360 measurements there are 105 deviations from the reference values, which is 29.17%, while in 70.83% of the measurements, the values of metabolic parameters are were within the reference values. The above results indicate that in practice a new way of reading and interpreting the results must be approached, which must take into account the position of the metabolic parameter value within the reference value, and not just the fact whether the metabolic parameter is outside the reference values.

Key words: cow, ketosis, metabolic profile, z-score.

UVOD

Ketoza predstavlja značajni metabolički poremećaj kod krava koji nastaje kao posledica negativnog energetskog bilansa, kada raste koncentracija ketonskih tela u krvi, mleku i urinu. Osnovne metaboličke adaptacije kod krava u ketozi su: povišena koncentracija BHB, povišena koncentracija NEFA, niža koncentracija glukoze, povišena vrednost jetrinih enzima i bilirubina, poremećaj makro i mirko elemenata, povišene vrednosti inflamatornih markera, oksidativni stres, insulinska rezistencija, a prate je i različite pridružene bolesti i poremećaji ponašanja. Ketoza krava je metabolička bolest koju se odlikuje poremećajem metabolizma ugljenih hidrata i masti sa povećanom proizvodnjom ketonskih tela u organizmu. Ova bolest nastaje kao posledica nemogućnosti kontrolisanja homeoretskih procesa i lošeg tolerisanja negativnog energetskog bilansa. Ketonska tela se normalno proizvode u organizmu zdravih krava (referentne vrednosti 0,2-0,6 mmol/L), ali je ketogeneza mnogo intenzivnija kod postojanja ketoze. Krave sa subkliničkom ketozom imaju vrednosti BHB više od 1,2 mmol/L, dok krave sa kliničkim znacima ketoze imaju koncentracije BHB koje su više od 2,6 mmol/L (1-3). Poznavanje vrednosti laboratorijskih parametara je od presudnog značaja za prepoznavanje patofizioloških i kliničkih efekata ketote. Obzirom da se ketoza često javlja u različitm formama, a da dobijene vrednosti metaboličkog profila kod većine parametara ostaju u okviru referentnihvrednosti, potrebno je ispitati varijabilnost krvnih parametara kako bi se preporučio novi način intepretacije metaboličkog profila u proceni ketoze kod krava.

Postoji velika potreba da se ispita stepen varijabilnosti ostlih krvnih parametara koji služe za procenu i evaluaciju težine ketoze kod krava, a mogu poslužiti i kao pomoćni dijagnostički i prognostički kriterijumi prilikom primene različitih terapijskih protokola. Pored navedenog, u radu ćemo predstaviti osnove kliničke patologije ketoze, sa posebnim aspektom na uticaj kliničko-patoloških procesa na vrednosti laboratorijskih dijangnostičkih parametara. Cilj ovog istraživanja je da se u nezavisnoj kazuističkoj analizi utvrdi stepen varijabilnosti krvnih parametara kod krava u ketozi. Očekujemo da ćemo utvrditi koji parametri pokazuju najveći stepen varijabilnosti, čime ćemo moći da damo praktični doprinos dijagnostici ketoze, jer ćemo utvrditi parametre koji su najsenzitivniji na ketozu, a čija varijabilnost u kombinaciji sa specifičnim promenama u vrednosti

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BHB omogućuju ne samo dijagnostiku ketoze već i graduisanje njene težine kod svake krave posebno.

MATERIJAL I METODE

U ogled je uključeno 45 krava koje su bile u različitim fazama ketoze u smislu klinička ketoza, subklinička ketoza, krave pod terapijom i u smislu perioda laktacije, zastupljene su krave u ranoj laktaciji i krave u piku laktacije kada se ova bolest najčešće javlja. Izvršen je klinički pregled, analiza ishrane, ocena telesne kondicije i prisustvo ketozi pridruženih bolesti. U obzir su uzete krave koje su barem jednom pre ovog uzorkovanja ili neposredno pre ovog uzorkovanja bile pozitivne na ketonska tela urinu, što je detektovano test trakama. Po zavrpenom odabiru uzimani su uzorci krvi. Uzorci krvi su uzimani venepunkcijom iz repne vene (*v.coccigea*) u serumske vakutajnere. Nakon toga, uzorci krvi su transportovani u Laboratoriju za patološku fiziologiju, Departman za veterinarsku medicinu, gde je posle centrifugiranja i izdvajanja seruma vršena biohemijska analiza krvi.

Određivanje metaboličkog profila – Prilikom analize metaboličkog profila krava, korišćene su fotometrijske reakcije i spektrofotometar Chemray proizvođača Rayto (Kina). Fotometrisanje je vršeno na talasnoj dužini i u vremenskom intervalu prema specifikaciji proizvođača. Korišćeni su standardni kitovi proizvođača Biosystems (Španija).

U cilju procene odstupanja najvažnijih biohemijskim parametara krvi u ketozi kod krava izvršeno je izračunavanje Z skora. Z skor predstavlja razliku dobijene vrednosti kod jedinke i srednje vrednosti populacije koja je podeljena sa vrednošću standardne devijacije. U ovom ogledu je korišćena srednja vrednost i standardna devijacije referentnih vrednosti kod krava koju su ustanivili autori iz Laboratorije za patološku fiziologiju i koji su objavnjeni u knjizi *Belić B., Cincović M. „Referentne vrednosti laboratorijskih parametara u krvi životinja“, Novi Sad, 2020*. Dobijeni reziltati su predstavljeni brojčano i grafički u tabeli. Pored brojčanih vrednosti radi lakše orijentacije izvršeno je i grafičko predstavnjanje varijabilnosti i to na tri načina: kazuistički (varijacija svih parametara u okviru jedne jedinke), sa aspekta vrednosti parametra, a posebno su izdvojeni parametri čiji je skor ispod odnosno iznad 2, što ukazuje da su vrednosti izvan referentnih vrednosti sa kojima u svakodnevnom radu poredimo ono što smo odredili u laboratoriji i na osnovu čega se donosi određeni zaključak. Koripšćen je *Microsoft Excel* i statistički paket *SPSS* za statističku obradu i prezentaciju podataka.

REZULTATI I DISKUSIJA

Rezultati istraživanja pokazuju da se sa intenzitetom ketoze kod krava može očekivati različit stepen odstupanja osnovnih parametara metaboličkog profila. Najveća vrednost odstupanja nađena je kod BHB, čiji je skor bio 6,15 (od 1,5 do 13,5). Skorovi odstupanja za ostale parametre iznosili su: za glukozu -0,04 (od -2,19 do 1,25), za holesterol 0,13 (od -2,42 do 3,57), za trigliceride -2,1 (od -3,13 do 1,13), za NEFA -0,06 (od -4,8 do 5,1), za alanin aminotransferazu -1,14 (od -3,2 do 2,6), za gama glutamil transferazu 1,36 (od -0,84 do 3,4), za ukupni bilirubin -0,64 (od -1,4 do 1,5) i za albumin -0,36 (od -3,67 do 5,10). Na osnovu prosečnih vrednosti odstupanja zaključujemo da se radi o veoma malim odstupanjima u odnosu na referentnu vrednosti za vrstu, osim za vrednosti BHB i trigliceride, koja su takva da se neće primetiti kao odstupanja od poznatih referentnih vrednosti (što se vidi poređenjem apsolutnih vrednosti sa utvrđnim referentnim vrednostima). Međutim, raspon vrednosti pokazuje da kod pojedinih životinja postoje visoka i niska odstupanja. Kod određene grupe životinja postoje visoke vrednosti pozitivnog odstupanja BHB iz pozitivno odstupanje vrednosti glukoze i holesterola i negativno ostupanje aspartat aminotransferaze, što je slika koja odgovara ketoznim krava koje su pod terapijom i koje uspešno odgovaraju na terapiju, a mogu se na osnovu apsolutnih vrenosti krvnih parametara klasifikovati i kao zdrave krave. Međutim kod ove grupe životinja skor odstupanja za trigliceride je dosta nizak, što znači da je jetra i dalje pati od akumulacije lipida, a verovatno je potrebno vreme da se akumulirani lipidi iz jetre razlože i da se jetra vrati u normalan funkcionalni status.U ovoj grupi krava je varijabilnost NEFA nestabilna, pa je kod nekih krava ispodprosečna, dok kod nekih krava i dalje prevazilazi i gornje referentne vrednosti, što dodatno objašnjava status triglicerida. Kod krava sa visokim vrednostima BHB postoje negativni skorovi za NEFA i albumin, što govori u prilog razvoju poremećaja funkcije jetre pod dejstvom ketonskih tela. Za razliku od aspartata koji značajno varira od slučaja do slučaja, vrednosti gama glutamil

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transferaze pokazuje pozitivna odstupanja koja mogu biti veća u zavisnosti od slučaja. Odstupanje bilirubina je bilo najmanje i kretalo se u rasponu jedne standardne devijacije, što ukazuje da je bilirubin parametar koji se ne menja ekstremno tokom ketoze. Kazuističko posmatranje govori u prilog tome da je potrebno posmatrati ne samo vrednosti određenih parametara nego i poziciju parametra u okviru referentnog opsega kako bi se zaključilo da li je i u kojoj meri ketoza pokrenula druge patofiziološke mehanizme. Ovo se posebno vidi kada se posmatra intenzitet odstupanja vrednosti u funkciji parametra. Nađeno je da sa porastom pozitivnog odstupanja BHB postoji statistički značajno negativno odstupanje vrednosti glukoze (-0,62; r<0,01), holesterola (-0,5; r<0,01), triglicerida (-0,38; r<0,01) i albumina (-0,40; r<0,01), a pozitivno odstupanje vrednosti NEFA (0,40; r<0,01), aspartata aminotransferaze (0,67; r<0,01), gama glutamil transferaze (0,06; NS) i ukupnog bilirubina (0,56; r<0,01). Navedene veze ukazuju da se težina stanja kod krave sa ketozom mora razmatrati određivanjem pozicije dobijene vrednosti u okviru referentne vrednosti nekog metaboličkog parametra. Našli smo da je odstupanje vrednosti bilirubina malo i ukoliko bismo želeli da posmatramo samo da li je bilirubin izvan ili u okviru referenetne vrednosti da bi ocenili kravu u kerozi, došli bi u zabludu jer je bilirubin gotovo kod svih jedinki u okviru referentnih vrednosti i to u okvirima prve standardne devijacije, ali se vidi da i te fine promene u okviru jedne devijacije koreliraju sa odstupanjem BHB. Svakodnevni praktični način procene zdravlja životinja ogleda se u poređenju dobijenih vrednosti sa referentnim vrednostima zbog toga je bilo potrebno da odredimo u kom procentu postoji veliko odstupanje vrednosti parametara (Z skor preko ili ispod 2), a koje u praktičnom smislu znači da je došlo do promene dobijenih vrednosti izvan referentnih. Kada se isključi BHB čije visoko odstupanje je u osnovi definicije ketoze i posmatraju se vrdnosti ostalih metaboličkih parametar, zaključujemo da u 360 merenja postoji 105 odstupanja od referentnih vrednosti, što je 29,17%, dok su u 70,83% merenja vrednosti metaboličkih parametara bili u okviru referentnih vrednosti. Navedeni rezultati ukazuju da se u praksi mora pristupiti novom načinu čitanja i interpretacije rezultata koje u obzir mora uzeti poziciju vrednostimetaboličkog parametra u okviru referentne vrednosti, a ne samo činjenicu da li se metabolički parametar nalazi izvan referentnih vrednosti. Zbog toga je potrebno kroz više terenske ali i strogo kontrolisane naučne studije utvrditi granične vrednosti parametara koje ukazuju da određena bolest postoji, čak iako se ta vrednost nalazi u okviru referente vrednosti.

Predviđanje nastanka ketoze i razlike u metaboličkoj adaptaciji utvrđeni su u jednom skorijem istraživanju (4). Cilj jednog rada bio je da se utvrdi razlika u metaboličkoj adaptaciji u prvoj nedelji posle teljenja kod zdravih krava i krava koji će razviti posle teljenja subkliničku ili kliničku ketozu tipa I i II. U ogled je uključeno 50 zdravih, 50 sa ketozom tipa I (3-6 nedelja) i 50 sa ketozom tipa II (1-3 nedelje posle teljenja) koje su izabrane retrospektivno. Metabolički markeri su određivani u prvoj nedelji pose teljenja, potom je za sve krave vršeno ispitivanje koncentracije BHB svaki drugi dan pomoću test traka (od kraja 1. do kraja 6. nedelje). Kod krava sa ketozom tipa I postojala je viša koncentracija NEFA i niža koncentracija insulina i glukoze u odnosu na kontrolnu grupu krava. Kod krava sa ketozom tipa II nađena je viša koncentreacija BHB, TNF-alfa i ukupnog bilirubina u odnosu na kontrolnu grupu. Vrednost RQUICKI indeksa je niža, a AST viša u krvi krava sa ketozom tipa II u odnosu na ketozu tipa I. Prediktivni značaj ovih markera koji su pokazali statistički značajno odstupanje ispitan je za svaku grupu krava formiranjem logističkih modela. Značaj modela je prikazan kroz area under curve (AUC) i iznosi: ketoza tipa I - (AUC=0,78±0,11); ketoza tipa II - (AUC=0,87±0,14); razlikovanje ketoze tipa I i II - (AUC=0,74±0,13). Udeo zajedničke varijanse markera koji su pokazali statistički značajno odstupanje i BHB u prvoj nedelji posle teljenja je značajno viši kod ketoznih (11,2-35,5%) nego kod zdravih krava (2,4-9,1%), što znači da metabolička adaptacija u funkciji ketogeneze nastaje rano posle partusa, pre razvoja pojavnih oblika ketoze.

Nastanak ketoze kod krava i njeno predviđanje ispitano je pomoću različitih modela. Svi modeli su pokazali da veliki značaj u predikciji ketoze imaju vrednosti NEFA, AST, glukoze i drugih parametara, sa čime se slažu naši rezultati. Nađeno je da granična vrednost od 0,26 mmol/L za NEFA pomaže u dijagnozi ketoze u prvih osam nedelja posle teljenja (5). Vrednosti iznad 0,76 mmol/L za NEFA, iznad 104 U/L za AST, ispod 140 U/L za holinesterazu i više od 3,3 µmol/L za ukupni bilirubin (6). Drugi autori (7) su potvrdili značaj glukoze, AST i NEFA u proceni razlikovanja zdravih i ketoznih krava (glukoza >3,04 mmol/L,

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AST <100 U/L, NEFA <0,82 mmol/L). U navedenim ogledima se radi o graničnim vrednostima u momentu kada je dijagnostikovana ketoza bez obzira na tip. Za razliku od ovih ogleda, u našem ogledu smo ispitali da li se na osnovu vrednosti krvnih parametara u prvoj nedelji posle teljenja može izvršiti predikcija nastanka ketoze. Poređenjem vrednosti AUC u ogledu i drugim ogledima koje smo spominjali zaključujemo da naš model (koji u obzir uzima vrednost parametara u prvoj nedelji posle teljenja) sa sličnom efikasnošću diskriminiše krave sa ketozom, kao i model koji uključuje pojedinačne krvne parametre (izmerene u momentu dijagnostike ketoze). Ovakav nalaz govori u prilog ranog prestrojavanja metabolizma krava ka nastanku ketoze. Klasifikovanje krava na osnovu lipolize, ketogeneze ili aktivnosti anaboličkih hormona u prvoj nedelji posle teljenja dovodi do metaboličkih razlika u ranoj laktaciji. Klasifikovanje krava na osnovu vrednosti lipolize i ketogeneze dovodi do razlika u metaboličkog adaptaciji. Potvrđeno je da krave sa višom koncentracijom BHB u prvom danu po teljenju (preko 1 mmol/L) imaju višu koncentraciju BHB, NEFA, AST i GGT i nižu koncentraciju IGF-I, posmatrano u prvih 14 nedelja posle teljenja (8). Rezultati domaćih autora su pokazali da kataboličko opterećenje i visoka ketogeneza dovode do opadanja vrednosti glukoze, porasta aktivnosti AST i ostalih metaboličkih promena u skladu sa nalazima u našem istraživanju (9). Kod krava u ketozi tipa I nađena je viša koncentracija BHB i NEFA i niža koncentracija insulina i glukoze u odnosu na kontrolnu grupu. Ketoza tipa I nastaje u periodu 3-6 nedelja posle teljenja najčešće kao posledica nedovoljnog unosa hrane u odnosu na proizvodne potrebe. Pokazan je značajan porast vrednosti NEFA, kao i opadanje koncentracije glukoze i insulina kod krava izloženih restrikciji hrane u ranoj laktaciji. Krave u ranoj laktaciji bile su u periodu 49±22 dana laktacije, što se slaže sa našim periodom dijagnostike ketoze tipa I, a to je 41±17 dana laktacije (10). Drugi autor u svom pregledu navodi da je masna jetra zapravo glavni pojavni oblik ketoze tipa II (11). Kao što je ranije diskutovano parametri jetre su značajni u dijagnostici ketoze kod krava. Utvrđeno je da su koncentracija BHB, NEFA, NEFA/holesterol i AST vrlo specifični u proceni nastanka masne jetre kod krava (12). Noviji rezultati pokazuju da kod krava u ketozi postoji viša koncentracija TNF-alfa (13, 14). Razlika između ketoze različitog tipa potvrđena je aspekta utinskih biohemijskih parametara, od kojih smo mnoge i mi koristili u našem ogledu (15). Hiperketonemija povećava koncentraciju TNF-alfa u kulturi monocita, kada se one kultivišu sa BHB (16). Visoka ekspresija TNF-alfa je regulisana interleukinima i u vezi je sa funkcionisanjem hepatocita (17). Poređenjem ketoze tipa I i II zaključujemo da je kod krava koje su razvile ketozu tipa II postojala viša aktivnost AST i niža vrednost RQUICKI indeka u odnosu na krave sa ketozom tipa I. Veća insulinska rezistencija (koja se ogleda u nižoj vrednosti RQUICKI ideksa) uz višu vrednost AST govori u prilog sklonosti ka nastanku ketoze tipa II. Utvrđena je viša koncentracija BHB, NEFA, glukoze, bilirubina, AST i ALT kod krava u ketozi tipa II u odnosu na ketozu tipa I. U jednom ispitivanju našli su nižu vrednost RQUICKI indeksa kod krava u ketozi u odnosu na krave kontrolne grupe (18). Naši autori su pokazali da indeksi insulinske rezistencije izmereni u bazalnim uslovima značajno utiču na dinamičke promene u vrednosti insulina, glukoze i NEFA kod krava u ketozi (19).

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Tabela 1. Varijabilnost Z skora u uslovima interakciji pojedinačnih slučajeva i parametara

	BHB (mmol/L)	Глукоза (mmol/L)	Холестерол (mmol/L)	Триглицериди (mmol/L)	NEFA (mmol/L)	AST (IU/L)	GGT (IU/L)	Ук.билирубин (µmol/L)	Албумин (g/L)
Средња вр.	0,60	3,00	3,90	0,35	0,60	80,00	15,00	8,00	38,00
SD	0,10	0,80	1,10	0,08	0,10	20,00	5,00	4,00	3,00
Zскор1	1,50	0,63	0,09	-0,75	-3,90	-2,82	2,78	-0,88	1,80
Zскор2	1,50	1,25	0,96	-2,63	-1,50	-2,55	0,82	-1,30	-1,20
Zскор3	0,10	0,00	-1,55	1,13	2,90	0,79	-0,58	-0,48	-0,47
Zскор4	1,60	-0,75	3,14	-1,50	-2,10	-2,02	1,76	-1,23	-0,53
Zскор5	1,60	0,13	-1,31	-2,88	0,00	-3,19	0,62	-0,53	-2,27
Zскор6	1,80	1,25	3,57	-1,88	-0,50	-2,48	2,26	-1,33	1,63
Zскор7	1,90	0,38	-0,36	-0,63	1,00	-2,65	1,38	-1,05	2,77
Zскор8	1,90	-0,13	1,77	-2,88	-0,10	-2,71	-0,18	-1,25	0,50
Zскор9	2,00	0,88	0,13	0,38	0,10	-2,27	3,40	-1,30	1,07
Zскор10	2,00	0,50	-0,73	-1,13	-1,50	-2,24	3,08	-1,20	3,43
Zскор11	2,00	-0,28	2,38	-2,50	-0,10	-2,44	1,02	-1,28	0,03
Zскор12	2,00	1,25	1,00	-2,75	0,00	-3,20	-0,86	-1,30	-0,80
Zскор13	2,20	0,50	2,17	-2,25	-2,00	-2,05	0,56	-1,30	-0,57
Zскор14	3,20	-0,05	0,96	-2,50	3,00	-1,51	1,16	-1,38	-0,67
Zскор15	3,60	1,25	1,45	-2,63	-2,00	-1,92	2,66	-1,38	0,93
Zскор16	4,90	0,50	0,32	-1,50	0,00	-2,49	2,02	-1,28	1,93
Zскор17	4,90	-0,50	1,91	-0,50	-0,50	-2,50	0,52	-1,25	-2,93
Zскор18	4,90	0,00	0,86	-2,13	0,00	-2,39	0,94	-0,73	-1,37
Zскор19	4,90	0,63	1,59	-2,00	-2,00	-2,25	1,00	-0,90	0,93
Zскор20	5,00	0,88	0,15	0,50	-2,40	-1,77	2,92	-1,23	5,10
Zскор21	5,00	1,25	1,15	-2,75	-3,50	-2,21	2,44	-1,30	0,90
Zскор22	5,50	0,25	0,09	0,25	-4,80	-2,44	1,24	-1,25	4,23
Zскор23	5,50	-0,35	0,84	-2,88	0,00	-2,43	1,42	-1,35	-0,10
Zскор24	5,60	0,38	-0,83	-2,25	-0,50	0,15	1,60	-1,23	-2,47
Zскор25	5,60	-0,38	1,30	-3,13	1,00	-2,23	1,54	-1,40	0,40
Zскор26	6,00	0,63	-1,70	-1,25	-4,10	-2,78	0,22	-1,23	-0,97
Zскор27	6,00	-0,45	0,30	-3,13	-4,60	0,10	1,26	1,00	-2,60
Zскор28	6,50	-0,66	-1,73	-2,75	-1,40	-2,37	0,70	0,50	0,13
Zскор29	6,80	-0,06	-1,51	-0,63	2,00	-1,13	2,32	0,25	-1,23
Zскор30	7,00	0,16	1,15	-2,50	1,10	2,00	0,62	0,75	-1,67
Zскор31	8,00	-1,00	0,01	-2,50	1,50	2,25	1,62	-1,28	-0,57
Zскор32	8,00	-0,75	0,40	-3,00	2,00	-1,25	1,62	-1,30	-0,97
Zскор33	9,00	-0,75	-2,42	-2,88	5,10	-1,58	1,28	1,40	-3,47
Zскор34	9,00	-0,19	-1,30	-2,88	0,90	1,45	0,62	0,25	-2,27
Zскор35	9,80	1,00	0,33	-1,75	2,90	1,05	1,58	-1,28	0,87
Zскор36	10,00	-0,50	-0,03	-2,25	-0,20	0,25	1,26	-1,33	-1,00
Zскор37	10,50	0,50	-0,15	-1,13	-1,50	-2,06	1,50	0,00	2,80
Zскор38	11,00	-1,13	-1,73	-2,88	-2,10	2,60	2,58	0,75	-3,67
Zскор39	11,00	-0,88	-1,31	-3,13	2,80	-1,50	-0,86	1,50	-0,97
Zскор40	11,50	-0,63	-0,59	-2,50	0,90	0,95	1,40	0,25	-3,67
Zскор41	12,00	-1,08	-1,17	-3,13	2,20	0,95	0,56	0,00	-3,33
Zскор42	13,00	-1,50	-1,73	-2,63	2,00	0,00	2,48	0,75	-0,03
Zскор43	13,50	-1,83	-1,04	-3,00	2,30	1,25	0,84	0,00	-1,07
Zскор44	13,70	0,25	0,23	-1,88	2,10	2,10	3,38	-1,10	-2,33
Zскор45	13,80	-2,19	-1,08	-3,13	2,90	0,00	0,84	0,50	-2,43

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ZAKLJUČAK

Na osnovu svega navedenog zaključujemo da je potrebno posmatrati ne samo vrednosti određenih parametara nego i poziciju parametra u okviru referentnog opsega kako bi se zaključilo da li je i u kojoj meri ketoza pokrenula druge patofiziološke mehanizme. Ovo se posebno vidi kada se posmatra intenzitet odstupanja vrednosti u funkciji parametra. Nađeno je da porastom pozitivnog odstupanja BHB postoji statistički značajno negativno odstupanje vrednosti glukoze, holesterola, triglicerida i albumina, a pozitivno odstupanje vrednosti NEFA, aspartata aminotransferaze, gama glutamil transferaze i ukupnog bilirubina. Navedene veze ukazuju da se težina stanja kod krave sa ketozom mora razmatrati određivanjem pozicije dobijene vrednosti u okviru referentne vrednosti nekog metaboličkog parametra. Svakodnevni praktični način procene zdravlja životinja ogleda se u poređenju dobijenih vrednosti sa referentnim vrednostima zbog toga je bilo potrebno da odredimo u kom procentu postoji veliko odstupanje vrednosti parametara (Z skor preko ili ispod 2), a koje u praktičnom smislu znači da je došlo do promene dobijenih vrednosti izvan referentnih. Kada se isključi BHB čije visoko odstupanje je u osnovi definicije ketoze i posmatraju se vrdnosti ostalih metaboličkih parametar, zaključujemo da u 360 merenja postoji 105 odstupanja od referentnih vrednosti, što je 29,17%, dok su u 70,83% merenja vrednosti metaboličkih parametara bili u okviru referentnih vrednosti. Navedeni rezultati ukazuju da se u praksi mora pristupiti novom načinu čitanja i interpretacije rezultata koje u obzir mora uzeti poziciju vrednostimetaboličkog parametra u okviru referentne vrednosti, a ne samo činjenicu da li se metabolički parametar nalazi izvan referentnih vrednosti. Zbog toga je potrebno kroz više terenske ali i strogo kontrolisane naučne studije utvrditi granične vrednosti parametara koje ukazuju da određena bolest postoji, čak iako se ta vrednost nalazi u okviru referente vrednosti.

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LITERATURA

1. *Oetzel G.R. Monitoring and testing dairy herds for metabolic disease. Vetrinary Clinics of North America: Food Animal Practice 2004, 20, 651-674.*
2. *Djoković R. Cincović M., Belić B., Toholj B., Davidov I. Hristovska T. Relationship between blood metabolic hormones, metabolites and energy balance in Simmental dairy cows during peripartum period and lactation. Pak Vet J 2015, 35, 2, 163-167.*
3. *Belić B., Cincović M., Lakić I., Đoković R., Petrović M., Ježek J., Starič J. Metabolic Status of Dairy Cows Grouped by Anabolic and Catabolic Indicators of Metabolic Stress in Early Lactation. Acta Scientiae Veterinariae 2018, 46, 1, 9.*
4. *Delić B., Belić B., Cincović M.R., Djokovic R., Lakić, I. Metabolic adaptation in first week after calving and early prediction of ketosis type I and II in dairy cows. Large Animal Review 2020, 26, 2, 51-55.*
5. *Nowroozi Asl A., Nazifi S., Rowshan Ghasrodashti A., Olyaee A. Prevalence of subclinical ketosis in dairy cattle in the Southwestern Iran and detection of cutoff point for NEFA and glucose concentrations for diagnosis of subclinical ketosis. Prev.Vet. Med. 2011, 100, 38-43.*
6. *Sun Y., Wang B., Shu S., Zhang H., Xu C., Wu L., Xia C. Critical thresholds of liver function parameters for ketosis prediction in dairy cows using receiver operating characteristic (ROC) analysis. Veterinary Quarterly 2015, 35, 3, 159-164.*
7. *Cao Y., Zhang J., Yang W., Xia C., Zhang H.Y., Wang Y.H., Xu C. Predictive value of plasma parameters in the risk of postpartum ketosis in dairy cows. J Vet Res 2017, 61, 91-95.*
8. *Kessel S., Stroehl M., Meyer H.H.D., Hiss S., Sauerwein H., Schwarz F.J., Bruckmaier R.M. Individual variability in physiological adaptation to metabolic stress during early lactation in dairy cows kept under equal conditions. J Anim Sci 2008, 86, 2903-2912.*
9. *Cincović M.R., Belić B., Radojičić B., Hristov S., Đoković R. Influence of lipolysis and ketogenesis to metabolic and hematological parameters in dairy cows during periparturient period. Acta veterinaria (Beograd) 2012, 62, 429-444.*

10. *Bjerre-Harpøth V., Friggens N.C., Thorup V.M., Larsen T., Damgaard B.M., Ingvartsen K.L., Moyes K.M. Metabolic and production profiles of dairy cows in response to decreased nutrient density to increase physiological imbalance at different stages of lactation. J Dairy Sci 2012, 95, 2362-2380.*
11. *Oetzel G.R. Herd-level ketosis–diagnosis and risk factors. In Proceedings of the 40th annual conference of bovine practitioners, Vancouver, Canada. pp. 67-91, 2007.*
12. *Mostafavi M., Seifi H.A., Mohri M., Jamshidi, A. Optimal thresholds of metabolic indicators of hepatic lipidosis in dairy cows. Revue de Médecine Vétérinaire 2013, 164, 12, 564-571.*
13. *El-Deeb W.M., El-Bahr S.M. Biomarkers of ketosis in dairy cows at postparturient period: acute phase proteins and pro-inflammatory cytokines. Veterinarski arhiv 2017, 87, 431-440.*
14. *Zhang G., Hailemariam D., Dervishi E., Goldansaz S.A., Deng Q., Dunn S.M., Ametaj B.N. Dairy cows affected by ketosis show alterations in innate immunity and lipid and carbohydrate metabolism during the dry off period and postpartum. Res Vet Sci, 2016, 107, 246-256.*
15. *Chuang X.U., Tai-yu S., Yuan Y.A.O., Hong-jiang Y., Cheng X.I.A., Hong-you Z. Blood clinicopathological differences between type I and II ketosis in dairy cows. Indian Journal of Animal Research 2016, 50, 753-758.*
16. *Jain S.K., Kannan K., Lim G., McVie R., Bocchini J. A. Hyperketonemia increases tumor necrosis factor-α secretion in cultured U937 monocytes and type 1 diabetic patients and is apparently mediated by oxidative stress and cAMP deficiency. Diabetes 2002, 51, 2287-2293.*
17. *Loor J.J., Dann H.M., Everts R.E., Oliveira R., Green C.A., Guretzky N.A.J., Rodriguez-Zas S.L., Lewin H.A., Drackley J.K. Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. Physiol. Genomics. 2005, 23, 217-226*
18. *Xu C., Shu S., Xia C., Wang B., Zhang H.Y., et al. Investigation on the Relationship of Insulin Resistance and Ketosis in Dairy Cows. J Veterinar Sci Technol 2014, 5, 162. doi:10.4172/2157-7579.1000162*
19. *Djoković R., Dosković V., Cincović M., Belić B., Fratrić N., Jašović B., Lalović M. Estimation of Insulin Resistance in Healthy and Ketotic Cows during an Intravenous Glucose Tolerance Test. Pakistan Veterinary Journal 2017, 37, 4.*

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POVEZANOST GLUKOZE, INSULINA, NEFA I INDEKSA INSULINSKE REZISTENCIJE SA METABOLIČKOM ADAPTACIJOM KRAVA U PERIODU PRE I POSLE TELJENJA

THE RELATIONSHIP OF GLUCOSE, INSULIN, NEFA AND INDEX OF INSULIN RESISTANCE WITH THE METABOLIC ADAPTATION OF COWS IN THE PERIOD BEFORE AND AFTER CALVING

Maja Došenović Marinković[a], Biljana Delić Vujanović[a], Marko Cincović[b], Siniša Grubač[c], Radojica Đoković[d]

[a]Academy of applied studies Šabac, Dobropoljska 5, 15000 Šabac, Serbia;
[b]University of Novi Sad, Faculty of Agriculture, Department of veterinary medicine, Novi Sad, Serbia
[c] Scientific veterinary institute Novi Sad, Serbia
[d]Faculty of Agronomy, Čačak, Serbija
*****Correspondence: maja_2511@yahoo.com

M.D.M. https://orcid.org/0009-0007-8904-0197, M.C https://orcid.org/0000-0002-5683-8983, S.G. https://orcid.org/0000-0002-8967-1358, R.Dj. https://orcid.org/0000-0003-0900-3227

SAŽETAK

Metabolički pokazatelji lošeg energetskog statusa krava su: snižena koncentracija glukoze i insulina i povišena koncentracija NEFA uz porast insulinske rezistencije. Ovakav metabolički profil karakterističan je za peripartalni period kod krava i nastaju usled smanjenog unosa hrane i povećanog usmeravanja glukoze ka mlečnoj žlezdi i gravidnom uterus. Cilj ovog istraživanja je da se ispitaju razlike u insulinskoj rezistenciji kod krava u zasušenju i ranoj laktaciji i ispita veza između pokazatelja insulinske rezistencije i parametara metaboličkog profila u ranoj laktaciji. Dobijena je statistički značajna korelacija između metaboličkih parametara i pokazatelja insulinske rezistencije, a ove veze su najizraženije u postpartalnom periodu. RQUICKI indeks ima vrlo slabu prediktivnu vrednost za metaboličke parametre, dok vrednosti insulina, glukoze i NEFA imaju značajnu prediktivnu vrednost za veliki broj parametara. Vrednosti NEFA i insulina u antepartalnom periodu koreliraju sa pojedinim metaboličkim parametrima, ali je broj statistički značajnih korelacija značajno manji. Pojedinačne vrednosti insulina, glukoze i NEFA imaju mnogo značajniju povezanost sa metaboličkim parametrima u odnosu na RQUICKI indeks insulinske rezistencije koji se izračunava iz navedena tri parametra.

Ključne reči: krave, metabolički status, insulinska rezistencija, glukoza, insulin, NEFA.

ABSTRACT

Metabolic indicators of poor energy status of cows are: decreased glucose and insulin concentration and increased NEFA concentration with increased insulin resistance. This metabolic profile is characteristic of the peripartum period in cows and is caused by reduced food intake and increased glucose directing towards the mammary gland and the pregnant uterus. The aim of this research is to examine the differences in insulin resistance in cows in dry and early lactation and examine the relationship between indicators of insulin resistance and metabolic profile parameters in early lactation. A statistically significant correlation was obtained between metabolic parameters and indicators of insulin resistance, and these relationships are most pronounced in the postpartum period. The RQUICKI index has a very weak predictive value for metabolic parameters, while the values of insulin, glucose and NEFA have a significant predictive value for a large number of parameters. The values of NEFA and insulin in the antepartum period correlate with certain metabolic parameters, but the number of statistically significant correlations is significantly lower. The individual values of insulin, glucose and NEFA have a much more significant association with metabolic parameters compared to the RQUICKI index of insulin resistance, which is calculated from the three parameters.

Key words: cows, metabolic status, insulin resistance, glucose, insulin, NEFA.

PERIPARTALNE METABOLIČKE ADAPTACIJE

Peripartalni period karakteriše negativan energetski bilans, potrošnja masti u energetske svrhe perifernog tkiva i potrošnja glukoze za potrebe vimena i proizvodnju mleka. Sve ovo uslovljava pad koncentracije glukoze i povećanje koncetracije neesterifikovanih masnih kiselina (NEFA). Preterana upotreba neesterifikovanih masnih kiselina dovodi do stvaranja ketonskih tela u jetri dok se u krvi povećava koncentracija beta hidroksibutirata (BHB). Ove metaboličke promene omogućavaju da glukoza (energija) bude preusmerena ka plodu i mlečnoj žlezdi. U ovom periodu periferna tkiva povećano koriste masti kao izvor energije. Da bi zadovoljila potrebe za glukozom, jetra troši rezerve glikogena. Kao posledica povećanog metabolisanja masti, stvaraju se i akumuliraju ketonska tela i trigliceridi. U peripartalnom periodu raste koncentracija oksitocina i estradiola i drugih polnih hormona, koji omogućuju ponovnu aktivaciju jajnika. Smanjena osetljivost na insulin i homeoretsko delovanje hormona raste, uz povećanu koncentraciju neesterifikovanih masnih kiselina i ketona dovode do smanjenog apetita kod krava (1). Sve ove metaboličke adaptacije nastaju u cilju podrske laktaciji. Sve hranljive materije se usmeravaju ka mlečnoj žlezdi. Mlečna žlezda koristi glukozu za proizvodnju laktoze, mlečnog šećera, koji je higroskopan, te povećava volumen proizvedenog mleka. Proces stvaranja mlečnih proteina se odvija upotrebom amino-kiselina iz krvi. Masne kiseline utiču na proizvodnju mlečne masti. Negativan energetski bilans kod krava dovodi do povećane koncentracije mlečne masti (zbog povećanog korišćena masnih metabolita iz krvi) i snižene koncentracije proteina (zbog manjka energije i nedostatka prekursora za proizvodnju dovoljne koncentracije proteina) (2). Optimalno snabdevanje vimena glukozom je neophodno za perzistentnu proizvodnju mleka. Kada bi se glukoza povećano trošila za potrebe perifernog tkiva, kao što je slučaj prilikom toplotnog stresa kod krava, došlo bi do znatnog smanjenja u proizvodnji i kvalitetu mleka (3). Mlečna žlezda ima prioritet prilikom upotrebe glukoze u organizmu krava, obzirom da je glukoza značajan prekursor laktoze. Mlečna žlezda u peripartalnom periodu povećano troši glukozu, jer poseduje receptore, koji su insulinnezavisni u procesu usvajanja glukoze u ćelije, u odnosu na ostalo periferno tkivo, koje je bogato insulinzavisnim receptorima čija gustina u laktaciji opada (4,5). Upravo je ovo uzrok za nastanak svih adaptacionih procesa, koji su prisutni u peripartalnom periodu. Rađena su istraživanja u kojima restrikcija hrane u periodu zasušenosti, nije pokazala uniformne rezultate. Izvođeni su ogledi u kojima restrikcija pokazuje pozitivan efekat na postpartalni unos hrane, proizvodnju mleka i metaboličku adaptaciju, međutim, u mnogim ogledima su dobijeni suprotni rezultati (6,7). Restrikcija hrane u periodu laktacije dovodi do pada u proizvodnji mleka i povećane lipidne mobilizacije. Nastaje ketogeneza uz pad koncentracije glukoze i promenjena senzitivost na insulin. Stadijum laktacije i mlečnost značajno utiču na adaptacionu sposobnost krava prilikom restrikcije hrane u toku laktacije (8).

Rezistencija na insulin je u humanoj populaciji okarakterisana metaboličkom acidozom, hiperglikemijom, smanjenom tolerancijom na glukozu, glukozurijom, ketonemijom, ketonurijom, pojačanom diurezom, hipovolemijom, dehidracijom, polidipsijom i depresijom centralnog nervnog sistema (9). Većina ovih poremećaja je uočena i kod preživara u uslovima indukovane ili spontane hepatične lipidoze (masne jetre) (10-12). Zapaženo je da su činioci odgovorni za nastanak insulinske rezistencije kod ljudi, isti ili slični onima, koji se dovode u vezu sa nastankom masne jetre i ketoze kod preživara. Ovi činioci uključuju kasni graviditet, gojaznost, hiperinsulinemiju, ishranu lipidima, hiperlipidemiju, pothranjenost, hormonalnu konstelaciju, genetske faktore i inflamaciju.

Metabolički pokazatelji lošeg energetskog statusa krava su: snižena koncentracija glukoze, povišena koncentracija NEFA i povišena koncentracija BHB. Ovakav metabolički profil karakterističan je za peripartalni period kod krava, pri čemu se kod većeg broja krava ovi parametri nalaze znatno preko referentnih vrednosti (13).

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Glukoza, NEFA i BHB su u korelaciji sa negativnim energetskim bilansom tokom perioda laktacije. Hipoglikemija kod krava nastaje usled smanjenog unosa hrane i povećanog usmeravanja glukoze ka mlečnoj žlezdi i gravidnom uterus (14). Vrednost glikemije pokazuje tendenciju akutnog pada u periodu oko partusa. Na početku laktacije potebe za glukozom se povećavaju. Smatra se da hranom krave mogu podmiriti oko 10% svojih potreba za glukozom, pa samim tim glukoneogeneza predstavlja ključni mehanizam održavanja glikemije u organizmu krava (15). Homeoretsku adaptaciju metabolizma glukoze u laktaciji predstavlja porast hepatične glukoneogeneze. U prepartalnom periodu propionat, laktat, alanin i glicerol u velikoj meri imaju uticaj na proizvodnju glukoze u jetri. Propionat vodi poreklo iz reakcija ruminalne fermentacije, laktat iz Korijevog ciklusa, aminokiseline potiču od katabolizma proteina, dok glicerol potiče iz katabolisanog masnog tkiva. U peripartalnom periodu njihovo učešće je sledeće: propionat u neto proizvodnji glukoze učestvuje 50-60%, amino-kiseline učestvuju 20-30%, laktat 15-20%, dok glicerol učestvuje 2-4% (16). Intravenskom aplikacijom propionata kod zdravih krava nastaje višestruki porast koncetracije glukoze (2-2,5 puta) kod zdravih krava u prvom satu nakon intravenske aplikacije. Kod nekih bolesti krava u kojima biva zahvaćena jetra, kao što je to slučaj kod ketoze, dolazi do značajno slabije produkcije glukoze. Upravo ovo, ukazuje na značaj jetre u glukoneogenetskom procesu (17). Upravo iz ovog razloga aplikacija propionata može biti od velike pomoći u optimizaciji metabolizma u peripartalnom periodu (18).

U peripartalnom periodu koncentracija glukoze obrnuto je proporcionalna koncentraciji NEFA i BHB. Neesterifikovane, slobodne masne kiseline su u cirkulaciji prisutne u vidu kompleksa sa albuminima. Predstavljaju alfa-lipoproteinsku frakciju. NEFA se sastoji od 12 slobodnih masnih kiselina. Među slobodnim masnim kiselinama su najzastupljenije palmitinska, stearinska, oleinska i linoleinska (17). Merenjem protoka palmitinske kiseline utvrđeno je da krave u peripartalnom periodu mogu da mobilišu i do 2,9 kg masti (19). Obezbeđivanje masnih kiselina kao izvora energije predstavlja najznačajniji homeoretski mehanizam u ranoj laktaciji. NEFA u krvi uglavnom vode poreklo iz lipidne mobilizacije koja nastaje u procesu katabolizma masnog tkiva, koja se javlja usled endokrinih izmena i smanjnog unosa hrane. Smanjen unos hrane sam po sebi nije dovoljan da pokrene postupak lipidne mobilizacije (20). Neesterifikovane masne kiseline mogu da se metabolišu u svim tkivima, stim što je težište na jetri koja je centralni organ metabolizma. Metabolički procesi u kojima učestvuje NEFA su: a) potpuna oksidacija masnih kiselina do vode i ugljenik-4-oksida, b) delimična oksidacija do acetil koenzima A i sinteza ketonskih tela (BHB), c) formiranje triglicerida iz NEFA u procesu resinteze i njihov transport iz jetre putem VLDL lipoproteina, d) resinteza triglicerida u jetri, njihova akumulacija i masna degeneracija jetre. NEFA i BHB su slabo varijabilni i shodno tome pokazuju veliki dijagnostički značaj u proceni metaboličkog i zdravstvenog statusa krava. Koncentracija glukoze je srednje varijabilna vrednost, te shodno tome ima srednju vrednost u dijagnostici. Koncentracija NEFA preko 0.4 mmol/l odnosno 0.8 mmol/l u prvoj nedelji nakon partusa i koncentracija BHB veća od 1,2 mmol/l u prvoj i/ili drugoj nedelji nakon partusa češće je prisutna kod krava kod kojih su prisutne peripartalne bolesti. Koncetracija glukoze se nije pokazala kao statistički značajan pokazatelj zdravlja i produktivnosti krava obzirom na njenu srednju varijabilnost. Njena orijentaciona vrednost ispod 2.3 mmol/l može da ukaže na nastanak različitih oboljenja (21-24). Adaptacioni kapacitet krava je u korelaciji sa glikemijom i koncentracijom NEFA u ranoj laktaciji. U slučaju da je koncentracija glukoze niska, intenziviraće se hipotalamo-hipofizni odgovor. Visoka koncentracija NEFA usloviće umanjen odgovor nadbubrega na ACTH. Sve navedene činjenice upućuju na zaključak da ovi metaboliti imaju uticaj na adaptacionu sposobnost krava u peripartalnom periodu (25).

POVEZANOST METABOLIČKIH PARAMETARA I FAKTORA INSULINSKE REZISTENCIJE

Cilj ovog istraživanja je da se ispitaju razlike u insulinskoj rezistenciji kod krava u zasušenju i ranoj laktaciji i ispita veza između pokazatelja insulinske rezistencije i parametara metaboličkog profila u ranoj laktaciji. Postavljene su sledeće hipoteze:-postoji značajna veza između koncentracije insulna, glukoze, NEFA i RQUICKI indeksa insulinske rezistencije kod krava u zasušenju i ranoj laktaciji; -postoji značajna veza između pokazatelja insulinske rezistencije i

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metaboličkog statusa krava u ranoj laktaciji; - ispitivanje mogućnosti da se rano tokom zasušenja prepoznaju krave koje mogu ući u veliku insulinsku rezistenciju u periodu rane laktacije, pa se na njih mora obratiti pažnja i uvesti posebne mere ishrane.

Ogled je izvršen na 30 krava Holštajn-frizijske rase u drugoj i trećoj laktaciji, bez znakova poremećaja zdravlja, uz proizvodnju mleka 7500±950 litara. Krave su gajene u slobodnom sistemu, na dubokoj prostirci, ali su u tranzicionom periodu bile u porodilištu, gde je postojao vezani sistem. Krave su hranjene miksovanim obrokom (TMR, total mixed ration) kojim se zadovoljavaju kompletne potrebe krava. U početku je u ogled bilo uključeno 40 krava, ali je 10 krava isključeno usled različitih peripartalnih problema (težak porođaj, inapetenca, ketoza, dugotrajno ležanje posle partusa, problem sa započinjanjem laktacije).

Krv je uzimana venepunkcijom *v.coccigea* kod krava u periodu pre jutarnjeg hranjenja,da bi se izbegao prandijalni efekat na vrednost metabolita. Krv je uzeta 4-6 nedelja pre teljenja (zasušenje) i 1-2 nedelje posle teljenja (rana laktacija). U uzorcima dobijenim tokom zasušenja određena je koncentracija glukoze, insulina, NEFA i izračunata je vrednost RQUICKI indeksa. U uzorcima krvi uzetim posle teljenja određena je vrednost parametara kao u zasušenju uz određivanje metaboličkog profila. Korišćene su fotometrijske reakcije i fotometar proizvođača *Rayto (RT1904c).*

Dobijena je statistički značajna korelacija između metaboličkih parametara i pokazatelja insulinske rezistencije u PP periodu. Metabolička adaptacija krava povezana je i sa intenzitetom promena vrednosti (delta vrednosti) pokazatelja insulinske rezistencije. Međutim, nisu utvrđene značajne korelacije između metaboličkiog profila PP i pokazatelja insulinske rezistencija AP. Korelacija između parametara i njihova statistička značajnost prikazane su u tabeli. Regresione analize (dijagram regresione linije i regresiona jednačina) za sve statistički značajne korelacije prikazane su u grafikonima. Uopšteno govoreći zaključujemo da PP vrednosti pokazatelja insulinske rezistencije i promena u njihovoj vrednosti (AP-PP) značajno utiču na vrednost metaboličkih parametara. RQUICKI indeks ima vrlo slabu prediktivnu vrednost za metaboličke parametre, dok vrednosti insulina, glukoze i NEFA imaju značajnu prediktivnu vrednost za veliki broj parametara. Pojedinačne vrednosti insulina, glukoze i NEFA imaju mnogo značajniju povezanost sa metaboličkim parametrima u odnosu na RQUICKI indeks insulinske rezistencije koji se izračunava iz navedena tri parametra.

Grafikon 1. Regresiona analiza povezanost pokazatelja insulinske rezistencije AP i metaboličkih parametara PP

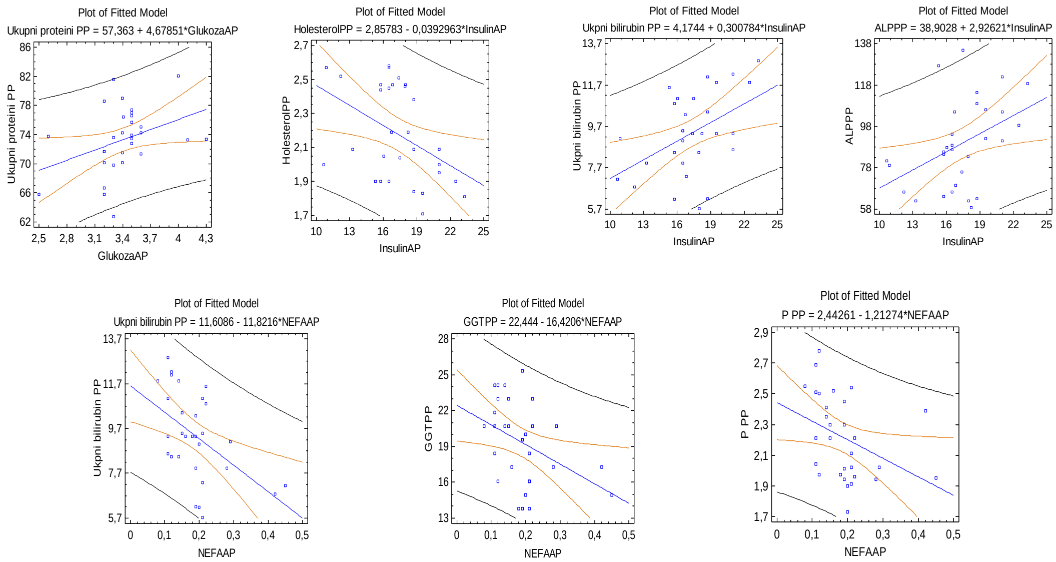

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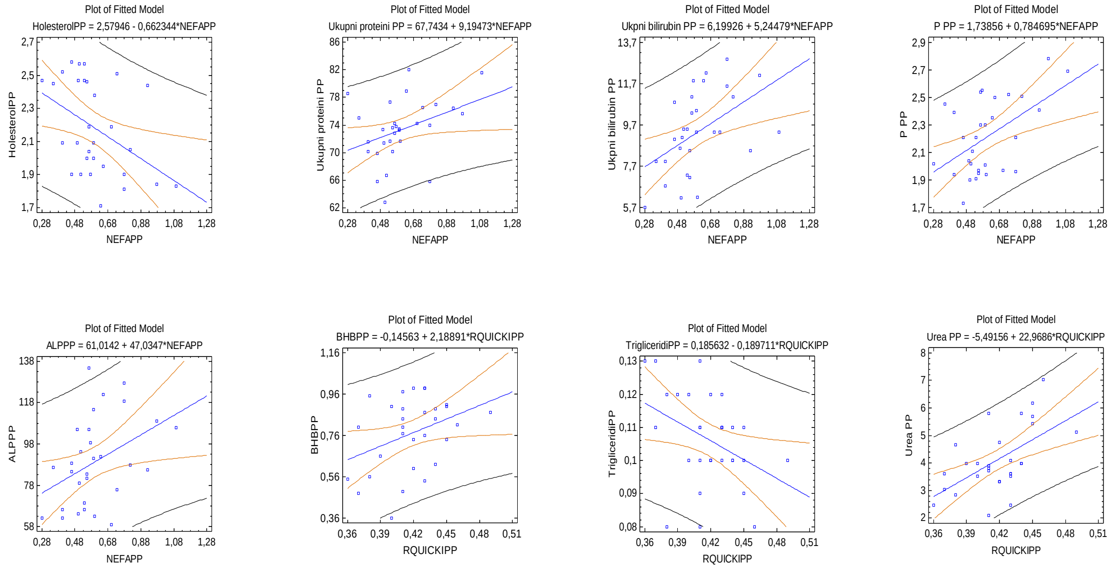
Tabela 1. Korelacija između vrednosti faktora insulinske rezistencije AP i PP i parametara metaboličkog profila PP

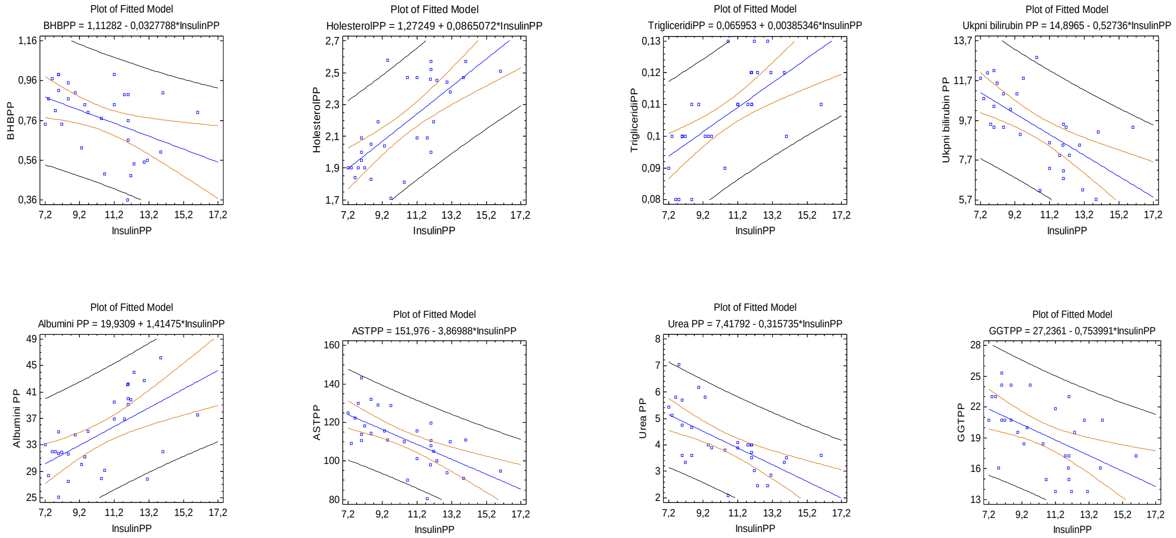
	Glukoza AP	Insulin AP	NEFA AP	RQUICKI AP	Glukoza PP	Insulin PP	NEFA PP	RQUICKI PP
BHBPP	0,2312[a]	0,0545	0,0507	-0,0409	-0,6528[a]	-0,4403	0,1841	0,3692
	0,2190[b]	0,7747	0,7903	0,8301	0,0001[b]*	0,0149*	0,3301	0,0446*
TrigliceridiPP	0,2379	-0,2828	0,1593	-0,0722	0,6612	0,6412	-0,3379	-0,3965
	0,2055	0,1299	0,4005	0,7046	0,0001*	0,0001*	0,0678	0,0301*
HolesterolPP	0,0586	-0,4225	0,2495	-0,0857	0,6225	0,7183	-0,4293	-0,3355
	0,7582	0,0200*	0,1836	0,6526	0,0002*	0,0000*	0,0179*	0,0699
Uk.bil. PP	-0,1325	0,4650	-0,5073	0,3422	-0,6402	-0,6296	0,4888	0,2372
	0,4853	0,0096*	0,0042*	0,0642	0,0001*	0,0002*	0,0061*	0,2070
ASTPP	-0,0084	0,2603	-0,2570	0,1258	-0,5475	-0,6428	0,2572	0,3597
	0,9649	0,1648	0,1703	0,5078	0,0017*	0,0001*	0,1701	0,0509
ALPPP	0,0748	0,4181	-0,3586	0,2065	-0,6286	-0,6288	0,4051	0,3025
	0,6946	0,0215*	0,0517	0,2735	0,0002*	0,0002*	0,0264*	0,1042
GGTPP	0,2463	0,3227	-0,4020	0,2580	-0,3678	-0,5136	0,3361	0,1964
	0,1895	0,0820	0,0277*	0,1686	0,0456*	0,0037*	0,0693	0,2984
Uk.prot. PP	0,3676	0,3057	-0,2975	0,0849	0,1572	-0,0917	0,3744	-0,2720
	0,0456	0,1004	0,1104	0,6555	0,4069	0,6297	0,0415*	0,1459
Albumini PP	0,0526	-0,3188	0,2689	-0,1374	0,4872	0,5913	-0,3457	-0,2460
	0,7825	0,0860	0,1507	0,4691	0,0063*	0,0006*	0,0613	0,1900
Urea PP	-0,1292	0,2144	-0,1645	-0,0224	-0,6483	-0,6288	0,0808	0,5744
	0,4963	0,2552	0,3851	0,9065	0,0001*	0,0002*	0,6714	0,0009*
CaPP	-0,0335	0,0182	-0,1254	0,0952	-0,0331	0,0180	-0,1252	0,0952
	0,8606	0,9239	0,5092	0,6170	0,8606	0,9239	0,5092	0,6170
P PP	-0,0400	0,2803	-0,3713	0,3013	-0,3157	-0,2713	0,5217	-0,0293
	0,8337	0,1335	0,0434*	0,1056	0,0893	0,1471	0,0031*	0,8778

a-koeficijent korelacije, b-statistička značajnost, *- statistički značajna korelacija

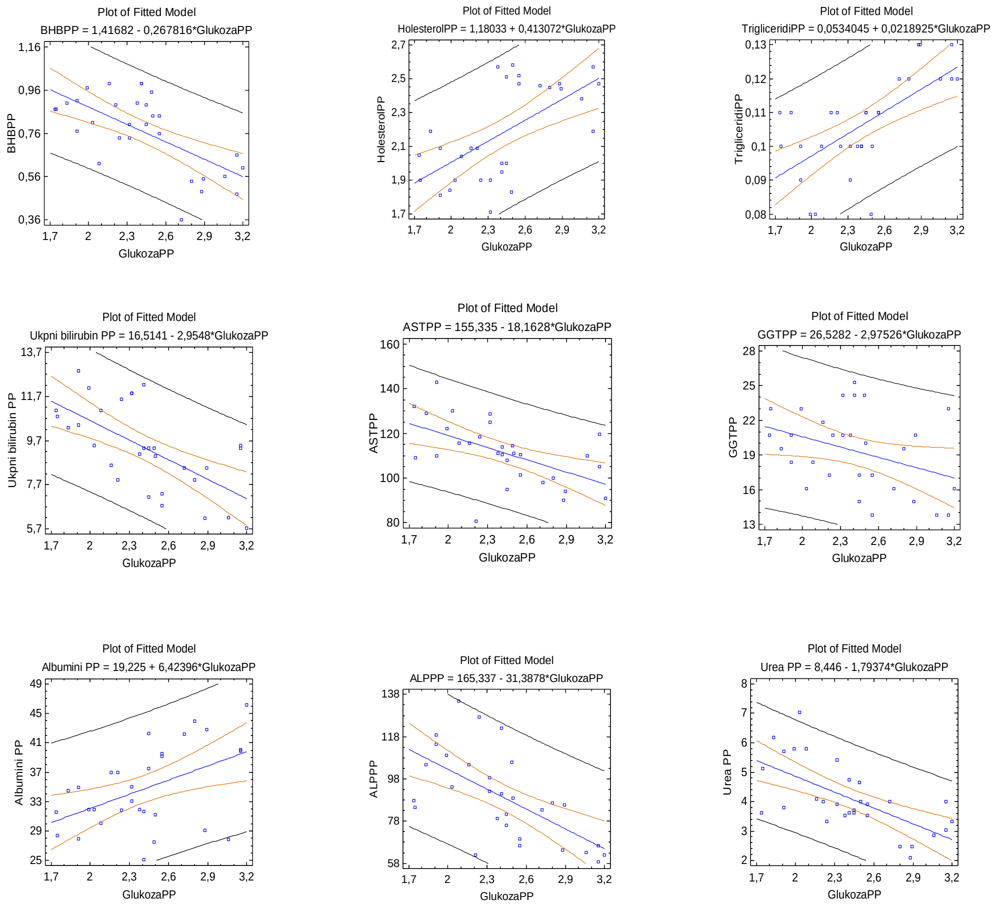
Grafikon 2. Regresione linije NEFA i RQUICKI PP parametara na metaboličke parametre



Grafikon 3. Regresione linije glukoze PP parametara na metaboličke parametre



Grafikon 4. Regresione linije glukoze PP parametara na metaboličke parametre



Sve promene koje su prisutne u metaboličkoj adaptaciji krava nastaju kao posledica njihove genetske predispozicije, telesne kondicije, ishrane i sastava hraniva u obroku. U periodu oko teljenja, veliku važnost ima stanje uhranjenosti krava odnosno telesna kondicija. Stanje uhranjenosti u značajnoj meri deluje uzajamno sa stepenom osetljivosti tkiva na insulin i/ili sposobnost krava da se adaptiraju na buduću laktaciju. Poremećaji energetskog metabolizma krava su najčešće posledica neadekvatne pripremljenosti životinja u periodu zasušenja na ono što sledi, a to je porast u proizvodnji mleka uslovljen početkom laktacije. Ovo se prevashodno odnosi na pozitivan bilans energije i preteranu gojaznost krava u prepartlnom periodu. Gojaznost se veoma često dovodi u vezu sa sposobnošću krava da se adaptiraju na povećane energetske potrebe na početku laktacije obzirom da se kod ugojenih krava smanjuje osetljivost tkiva na insulin (26). Gojazne krave imaju smanjen apetit u periodu oko teljenja (27). U takvim uslovima negativnog energetskog bilansa nedostatak energije se nadoknađuje iz sopstvenih rezervi, prevashodno lipomobilizacijom. Smanjenje apetita se dalje produbljuje, obzirom da sa porastom lipomobilizacije raste i koncentracija NEFA. Nekontrolisana mobilizacija masnih kiselina iz telesnih depoa može postati samoodrživ proces kada NEFA počne uticati na sekreciju insulina u beta ćelijama pankreasa i na insulinsku senzitivnost. Njihova oksidacija deluje na centar za glad. Centar za glad dobija signal da je jetra dobro snabdevena energijom iako se organizam nalazi u stanju negativnog energetskog bilansa, koji pokušava kompenzovati mobilizacijom telesnih rezervi (28). Obzirom da se maksimalno konzumiranje hrane uspostavlja tek nakon 10 do 15 dana od teljenja, period rane laktacije upravo karakteriše negativan bilans energije i posledično slabljenje kondicije i smanjenje masnog tkiva. Dakle, smanjena ili suprimirana funkcija beta ćelija pankreasa je jedan od uzroka koji može izazvati razistenciju. Promene na nivou receptora takođe mogu biti uzrok smanjenja inulinskog odgovora ali i promene na postreceptorskom nivou, kad nastaju greške u transdukciji insulinskog signala i/ili translokaciji GLUT molekula na membranama ciljnih ćelija (29). Insulin je pozitivan regulator procesa transkripcije i translokacije GLUT 4 molekula u insulin zavisnim tkivima. Razlika u nivou ekspresije GLUT 4 molekula na membranama mišićnih ćelija različitih jedinki nastaje upravo kao posledica razlike u vrednosti insulina u krvi. Kod gojaznih krava se zapaža znatno veća koncentracija glukoze u krvi, što ukazuje na poremećaje na nivou translokacije GLUT 4 molekula na membranama mišićnih ćelija kod ovih jedinki. Ovakav nalaz se može zapaziti i kod ljudi obolelih od dijabetesa tip 2 (30). On je primarni razlog smanjene senzitivnosti tkiva na insulin. Smanjena efikasnost insulina u procesu stimulisanja iskorišćavanja glukoze od strane perifernih tkiva se tumači time da se kod krava koje su u periodu zasušenja hranjene većom količinom krmnih smeša i / ili ugojenih krava poremećaji metabolizma glukoze posledica dugotrajne hiperinsulinemije koja u toku perioda zasušenja mehanizmom negativne povratne sprege reguliše ekspresiju protein insulinskih receptora. Više masne kiseline i inflamatorni citokini (TNF alfa i IL-6) su na početku laktacije kod gojaznih krava prisutni u većim koncentracijama u krvi i mogu uticati na prenošenje insulinskog signala, samim tim i na regulaciju metaboličkih procesa u ćelijama (31,32). Masno tkivo infiltrovano makrofagima je znatno aktivnije u proizvodnji TNF-alfa i drugih proinflamatornih citokina. Citokini blokiraju insulinske signalne puteve u hepatocitima. Kod gojaznih jedinki se proces lipomobilizacije odvija intenzivnije u odnosu na stvarne energetske potrebe u uslovima NEB. Ovakav slučaj je i kod ljudi obolelih od dijabetesa tip 2 (33). U uslovima insulinske rezistencije, smanjena je mogućnost korišćenja nepotrebno mobilisanih većih količina masnih kiselina u telesnim tkivima, prvenstveno mišićnom. Zato se suvišne masne kiseline usmeravaju ka jetri koja je odgovorna za regulaciju energetskog metabolizma i prometa masnih kiselina. Ovo ima za posledicu smanjeno iskorišćavanje energije i pojačavanje NEB (34). Ovo je razlog veće mogućnosti za nastanak masne infiltracije jetre kod gojaznih krava. Promene vrednosti glukoze posle teljenja u vidu njenog pada, je u pozitivnoj korelaciji sa koncentracijom glukoze pre teljenja. Ovo nam ukazuje da će kod krava sa višom glikemijom u periodu pre teljenja , biti intenzivniji pad posle teljenja. Obzirom da ketonska tela predstavljaju alternativni izvor energije za organe, njihov blagi porast u krvi predstavlja normalan mehanizam adaptacije na NEB u ranoj laktaciji (35). BHBA predstavlja dominantnu formu ketonskih tela, i njegova koncetracija u krvi predstavlja indeks oksidacije masnih kiselina (36). Kao posledica smanjenja energije a prisustva veće koncentracije proteina u obroku, dolazi do povećanja koncentracije uree. Usled energetskog deficita dolazi i do povećane mobilizacije masnih kiselina koje dospevaju u jetru i uzrokuju njeno metaboličko opterećenje praćeno porastom koncentracije ukupnog bilirubina. Hipoglikemija, hipoalbuminemija i hipoholesterolemija ukazuju na smanjenu sintetsku sposobnost jetre, dok znatno povećanje koncentracije ukupnog bilirubina i aktivnost AST u serumu ukazuje na oštećenje jetre i narušen morfološki integritet hepatocita (37). Ove promene se javljaju kao posledica akumulacije lipida u hepatocitima koja je proizvod povećane upotrebe masti u energetske svrhe usled neadekvatne ishrane i negativnog energetskog bilansa. Jetra je glavni organ koji učestvuje u adaptaciji metabolizma pa izmenjene vrednosti ovih parametara upravo upućuju na metaboličko opterećenje krava. Usled promena u metabolizmu kao posledica smanjenog unosa proteina putem hrane i pojačanog katabolizma proteina, dolazi do smanjenja ukupnih proteina u serumu sveže oteljenih krava. Albumini se tokom puerperijuma prevashodno koriste za sintetske procese, te je njihova koncentracija u krvi u tom periodu smanjena (38). Koncentracija albumina u krvi često prati koncentraciju proteina (39). U ranoj laktaciji se povećava unošenje proteina putem hrane, što posledično utiče na porast koncentracije albumina. Porast albumina nije odmah značajan nakon porasta proteinemije kod krava, obzirom da je za sintezu proteina potrebno izvesno vreme. Zato se smatra da urea predstavlja trenutni pokazatelj unosa proteina hranom a albumini su pokazatelj ne trenutnog, nego dugoročnog proteinskog statusa krava. U periodu posle teljenja se kao posledica povećanja koncentracije parathormona i povećane osetljivosti kostiju na njegovo dejstvo odvija povećana mobilizacija Ca iz kostiju pri čemu on biva transportovan u mleko.U istraživanju je dobijena očekivano niža vrednost koncentracije Ca koja je upravo uslovljena smanjenim unosom hranljivih materija na početku laktacije i smanjenom resorpcijom Ca u crevima, kao i izlučivanjem putem mleka. Dakle, koncentracija Ca u krvi opada sa otpočinjanjem laktacije, što su utvrdili i drugi istraživači (40). Pad koncetracije triglicerida i porast koncetracije NEFA u krvi su znak intenzivnog metabolizma masnih kiselina koji dovodi do posledičnog zamašćenja jetre. Ukoliko je nakupljanje triglicerida intenzivno, oni ometaju i potpuno blokiraju rad hepatocita. Dakle, upravo je energetski bilans taj koji je ključ adaptacije metabolizma u peripartalnom periodu. S toga ishranu u ovom periodu treba prilagoditi energetskim potrebama jedinki sa hranivima, koja omogućavaju lako usvajanje energije i sprečiti njen pad nakon partusa da bi metabolizam bio izbalansiran.

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LITERATURA

1. *Ingvartsen K.L., Andersen H.R. Integration of Metabolism and Intake Regulation: A Review Focusing on Periparturient Animals. J.Dairy Sci. 2000, 83: 1573-1597.*
2. *Šamanc H., Kirovski D., Dimitrijević B., Vujanac I., Damnjanović Z., Polovina M. Procena energetskog statusa krava u laktaciji određivanjem koncentracije organskih sastojaka mleka. Veterinarski glasnik 2006, 60(5-6): 283-297.*
3. *Belić B., Cincović M.R., Popović-Vranješ Anka, Pejanović R., Krajinović M. Metaboličke promjene i iskorištavanje metabolita u proizvodnji mlijeka kod krava u toplinskom stresu. Mljekarstvo 2011, 61(4): 309-318.*
4. *Komatsu T., Itoh F., Mikawa1 S., Hodate K. Changes in gene expression of glucose transporters in lactating and nonlactating cows. J. Anim. Sci. 2005, 83: 557–564.*
5. *Cant J.P., Trout D.R., Qiao F., Purdie N.G. Milk synthetic response of the bovine mammary gland to an increase in the local concentration of arterial glucose. J. Dairy Sci. 2002, 85, 494–503.*
6. *Mashek DG, Grummer RR. The ups and downs of feed intake in prefresh cows. Proc Four-State Nutr Conf LaCrosse, WI. MidWest Plan Service publication MWPS- 4SD16. pp. 153–158, 2003.*
7. *Dann H.M., Morin D.E., Bollero G.A., Murphy M.R., Drackley J.K. Prepartum Intake, Postpartum Induction of Ketosis, and Periparturient Disorders Affect the Metabolic Status of Dairy Cows. J. Dairy Sci. 2005, 88:3249–3264.*

ISBN 978-86-7520-611-8

8. Bjerre-Harpøth V., Friggens N.C., Thorup V.M., Larsen T., Damgaard B.M., Ingvartsen K.L., Moyes K.M. Metabolic and production profiles of dairy cows in response to decreased nutrient density to increase physiological imbalance at different stages of lactation. J.Dairy Sci 2012, 95(5): 2362-2380.
9. McCance K.L., Huether S.E. Alterations of hormonal regulations. Pathophysioilogy 2nd edn., Mosby, St Louis, pp. 674–692, 1994.
10. DeBoer G., Trenkle A., Young J.W. Glucagon, insulin, growth hormone and some blood metabolites during energy restriction ketonemia of lactating cows. J Dairy Sci 1985, 68:326–337.
11. Veenhuizen J.J., Drackley J.K., Richard M.J., Sanderson T.P., Miller L.D., Young W.J. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. J Dairy Sci 1991, 74:4238–4253.
12. Drackley J.K. Biology of dairy cows during the transition period: the final Frontier. J Dairy Sci 1999, 82:2259–2273.
13. Cincović M.R., Belić B., Vidović B., Krčmar LJ. Reference values and frequency distribution of metabolic parameters in cows during lactation and in pregnancy. Contemporary agriculture 2011, 60(1-2): 175-182.
14. Bell A., Bauman D. Adaptations of glucose metabolism during pregnancy and lactation. J. Mammary Gland Biol. Neoplasia 1997, 2(3): 265-278.
15. Đoković R.D. Endokrini status mlečnih krava u peripartalnom periodu. Agronomski fakultet, Čačak, 2010.
16. Reynolds C.K., Aikman P.C., Lupoli B., Humphries D.J., Beever D.E. Splanchnic metabolism of dairy cows during the transition fromlate gestation through early lactation. J. Dairy Sci. 2003, 86:1201–1217.
17. Đoković R., Šamanc H., Nikolić Z., Bošković-Bogosavljević S. Changes in blood values of glucose, isnulin and inorganic phophorus in healthy and ketotic cows after intravenous infusion of propionate solution. Acta Veterinaria Brno 2007, 76:533-539.
18. Overton T.R., Waldron M.R. Nutritional management of transition dairy cows: strategies to optimize metabolic health. J Dairy Sci 2004, 87:(E.Suppl.):E105–E119.
19. Husvéth F., Karsai F., Gaal T. Peripartal fluctuations of plasma and hepatic lipid components in dairy cows. Acta Vet Acad Sci Hung 1982, 30(1-3): 97-112.
20. Friggens N.C. Body lipid reserves and the reproductive cycle: towards a better understanding. Livest. Prod. Sci. 2003, 83: 219-236.
21. Roberts T., Chapinal N., LeBlanc S.J., Kelton D.F., Dubuc J., Duffield T.F. Metabolic parameters in transition cows as indicators for early-lactation culling risk. J. Dairy Sci. 2012, 95(6): 3057-3063.
22. Ospina P.A., Nydam D.V., Stokol T., Overton T.R. Associations of elevated nonesterified fatty acids and β-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J. Dairy Sci. 2010, 93(4): 1596-1603.
23. Ospina P.A., Nydam D.V., Stokol T., Overton T.R. Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J. Dairy Sci. 2010, 93(20): 546-554.
24. Nowroozi Asl A., Nazifi S., Rowshan Ghasrodashti A., Olyaee A. Prevalence of subclinical ketosis in dairy cattle in the Southwestern Iran and detection of cutoff point for NEFA and glucose concentrations for diagnosis of subclinical ketosis. Prev.Vet. Med. 2011, 100(1): 38-43.
25. Beerda B., Kornalijnslijper J.E., van der Werf J.T.N., Noordhuizen-Stassen E.N., Hopster H. Effects of Milk Production Capacity and Metabolic Status on HPA Function in Early Postpartum Dairy Cows. J. Dairy Sci. 2004, 87: 2094–2102.
26. Rukkwamsuk T., Wensing T., Geelen M.J. Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. J Dairy Sci 1998, 81:2904–2914.
27. Ingvartsen K.L., Andersen J.B. Integration of metabolism and intake regulation: a review focusing on periparturient animals. J Dairy Sci 2000, 83: 1573–1597.
28. Emery R.S., Liesman J.S., Herdt T.H. Metabolism of long chain fatty acids by ruminant liver. J Nutr 1992, 122: 832-837.
29. Vernon RG., Clegg RA., Flint DJ. Metabolism of sheepadipose tissue during pregnancy and lactation. Adaptation and regulation. Biochem. J. 1981, 200, 307-314.
30. Zierath J.R., Krook A., Wallberg-Henriksson H. Insulin actionin skeletal muscle from patients with NIDDM. Mol Cell Biochem 1998, 182:153-160.
31. Oikawa S., Oetzel G.R. Decreased insulin response in dairy cows following a four day fast to induce hepatic lipidosis. J Dairy Sci 2006, 89:2999-3005.
32. Pires J.A.A., Souza H., Grummer R.R. Induction of hiperlipidemia by intravenous infusion off tallow emulsion causes insulin resistance in Holstein cows. J Dairy Sci 2007, 90:2735-2744.

ISBN 978-86-7520-611-8

33. *Jin H.B., Gu Z.Y., Yu C.H., Li Y.M. Association of nonalcoholic fatty liver disease with type -2 diabetes: clinical features and independent risk factors in diabetic fatty liver patients. Hepatobiliary Pancreat Dis Int 2005, 4:389-392.*
34. *Blaak E.E. Fatty acid metabolism in obesity and type 2 diabetes mellitus. Proc Nutr Soc 2003, 62: 753-760.*
35. *McArt J.A.A, Nydam D.V, Oetzel G.R. Dry and parturient predictors of early lactation of hyperketonemia in dairy cattle. Journal of Dairy Science 2012c, 96:198-209.*
36. *Puppel K., Kuczyńska B. Metabolic profiles of cow's blood; a review. Journal of Science of Food and Agriculture 2016, doi: 10.1002/jsfa.7779.*
37. *Kalaitzakis E., Roubies N., Panousis N., Pourliotis K., Kaldrymidou E., Karatzias H. Evaluation of ornitin carbamoyl transferase and other serum and liver-derive analytes in diagnosis of fatty liver and postsurgical outcomeof left –displaced abomasums in dairy cows, J.Am Vet Med Assoc. 2006, 229, 1463-71.*
38. *Lofthammer K.H. Posledice neizbalansiranog obroka na zdravlje I reprodukciju krava (Einfusse und Folgen unausgeglichener Futterung auf Gesundheit und Fruchtarkeit des Milscrindes). Zbornik predavanja XX seminara za inovacije znanja veterinara , 71-120 Beograd, 1991.*
39. *Kaneko J.J., Harvey J.W., Bruss M.: Clinical biochemistry of domestic animals. Ed.6th, Academic Press, 2008.*
40. *Goff J.P., Horst R.L. Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci 1997, 80, 1260-1268.*

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Article
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LIPIDEMIA AND LIPID CONTENTS IN THE LIVER IN HOLSTEIN COWS DURING TRANSITION PERIOD

LIPIDEMIJA I SADRŽAJ LIPIDA U JETRI KOD HOLSTEIN KRAVA U PERIPARTALNOM TRANZIVIONOM PERIODU

Radojica Djoković[a*], Miloš Ži. Petrović[a], Biljana Andjelić[b], Aleksandar Čukić[c]

[a]Department of Animal Science, Faculty of Agronomy – Čačak, University of Kragujevac, Cara Dušana 34, Čačak, Serbia
[b]Department of Animal Science, Faculty of Agriculture – Kruševac, University of Niš, Kosančićeva 4, Kruševac, Serbia
[c]Department of Animal Science, Faculty of Agroculture – Lešak, University of Priština, Jelene Anžujske bb.Dušana 34, Serbia.
*Corresponding author: radojicadjokovic@gmail.com

R.Dj. https://orcid.org/0000-0003-0900-3227, M.Ž.P. https://orcid.org/0000-0002-4707-6796, B.A. https://orcid.org/0000-0002-3976-0539, A.Č. https://orcid.org/0000-0001-9063-3431

ABSTRACT

The aim of the present study was to determine a correlation between blood concentration of lipids and the content of lipids in the liver of dairy cows in the transitional period. The Holstein dairy cows (n = 40) were divided into four groups: the first group (A) included late pregnant cows (n = 10) from the 10th to 4th day before calving; the second group (B) included late pregnant cows (n = 10) from the 4th to 1st day before calving; the third group (C) included clinically puerperal healthy cows (n = 10), whereas the fourth group (D) included puerperal ketotic cows (n = 10). The liver and blood samples were taken from all the cows. Pathohistological examination of liver samples showed statistically significantly higher ($p < 0.01$) lipid infiltration in ketotic cows compared to healthy cows in late pregnancy and puerperium. Biochemical examination of blood serum showed significantly higher values ($p < 0.01$) of nonesterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) in ketotic cows, such as lower blood concentrations of glucose ($p < 0.01$), triacylglycerols (TG) ($p < 0.01$), and total cholesterol TChol. ($p > 0.05$) compared to the values obtained in the blood serum in the groups of healthy cows before and after calving. The significantly positive correlations were determined between the content of lipids in the liver and blood concentration of NEFA ($r = 0.67$; $p < 0.05$) and BHB ($r = 0.55$; $p < 0.05$) as well as the negative ones between the content of lipids in the liver and blood concentrations of glucose ($r = -0.45$; $p < 0.05$), TG ($r = -0.55$; $p < 0.05$) and TChlol. ($r = -0.39$; $p < 0.05$). Our investigations suggested that changes in the blood concentrations of NEFA, BHB, TG, TChol. and glucose served as major biochemical indicators in determining ketosis and liver steatosis in the dairy cows in the transitional period.

Key words: cows, fatty liver,ketosis, transitional period, biochemical indicator.

SAŽETAK

Cilj ovog istraživanja bio je da se utvrdi korelacija između koncentracije lipida u krvi i sadržaja lipida u jetri mlečnih krava u prelaznom periodu. Holštajn mlečne krave (n = 40) podeljene su u četiri grupe: prva grupa (A) obuhvatala je kasno steone krave (n = 10) od 10. do 4. dana pre teljenja; druga grupa (B) obuhvatala su kasno steone krave (n = 10) od 4. do 1. dana pre teljenja; treća grupa (C) obuhvata klinički puerperalne zdrave krave (n = 10), dok četvrta grupa (D) uključuje puerperalne ketotične krave (n = 10). Od svih krava uzeti su uzorci jetre i krvi. Patohistološki pregled uzoraka jetre pokazao je statistički značajno veću (p <

0,01) lipidnu infiltraciju kod ketotičnih krava u odnosu na zdrave krave u kasnoj gravidnosti i puerperijumu. Biohemijsko ispitivanje krvnog seruma pokazalo je značajno veće vrednosti (p < 0,01) neesterifikovanih masnih kiselina (NEFA) i beta-hidroksibutirata (BHB) kod ketotičnih krava, kao što su niže koncentracije glukoze u krvi (p < 0,01), triacilglicerola (TG) (p < 0,01), i ukupni holesterol TChol. (p > 0,05) u poređenju sa vrednostima dobijenim u krvnom serumu u grupama zdravih krava pre i posle teljenja. Utvrđene su značajno pozitivne korelacije između sadržaja lipida u jetri i koncentracije NEFA u krvi (r = 0,67; p < 0,05) i BHB (r = 0,55; p < 0,05), kao i negativne između sadržaja lipida. u jetri i koncentraciji u krvi glukoze (r = -0,45; p < 0,05), TG (r = -0,55; p < 0,05) i TChlola. (r = -0,39; p < 0,05). Naša istraživanja su pokazala da promene u koncentraciji NEFA, BHB, TG, TChol u krvi. a glukoza je služila kao glavni biohemijski indikatori u određivanju ketoze i steatoze jetre kod mlečnih krava u prelaznom periodu.

Ključne reći: krave, masna jetra, tranzicioni period, biohemijski indikatori.

INTRODUCTION

Production diseases i.e. diseases associated with improper nutrition or management are common in dairy cows and mostly occurred during transition period. (1-3). Ketosis is a common disease in high producing dairy cows during the early lactation period. Ketoss can be categorized into three types: ketosis type I, (spontaneous, underfeeding ketosis, 3-6 weeks after calving), ketosis type II or fatty liver (1-2 weeks postpartum), and ketosis type III, butyric acid silage (4-6). Subclinical ketosis may be diagnosed when serum BHB concentrations are above 1.2 mmol/l , and clinical ketosis with blood BHB level above 2.6 mmol/l (4,5). The transitional period in dairy cows included 3 weeks before and 3 weeks after calving, when metabolic processes were adapted to providing energy and nutrients required for the synthesis of milk compounds (1). Early lactation in dairy cows resulted in negative energy balance, high mobilization of lipids from bodily fat reserves as well as hypoglycaemia, ketonemia and ketouria (7-9). The main blood markers of lipomobilization in dairy cows are BHB, the most important ketone body,and NEFA (9-11). Lipid mobilisation characterised by highly concentrated NEFA in blood starts in a high degree of pregnancy, reaching its maximum in early lactation. NEFA are reesterified and accumulated in the form of TG in the liver, primarily due to the decreasing capacity of hepatocytes for transport of lipids by very low density lipoproteins (VLDL), (12). As a result, lipid mobilisation intense ketogenosis and lipogenesis in the liver and consequently lower concentrations of glucose, TG and T.Chol. in blood were manifested (13,12,11,14). Primary homeorhetic adaptation of glucose metabolism in early lactation leads to increased gluconeogenesis in the liver to direct glucose into the mammary gland for lactose synthesis (2). If the degree of gluconeogenesis in the liver does not meet the increased needs of glucose in dairy cows in early lactation, hypoglycaemia, lipidemia, ketonaemia and ketonuria are likely to occur (9,15). Liver can be categorized into normal liver or mild (0-20% of lipids), moderate (20-40% of lipids) and severe fatty liver (more than 40% of lipids) as dependent on the degree of pathology) (16,17,14) and a mild fatty infiltration of liver in dairy cows during transition and maximum lactation is considered to be almost physiological. However, when an important steatosis occurs, the endogenous liver syntheses are lowered, leading to decreases in blood concentrations of glucose, proteins, lipids and urea (12,18). The aim of the present study was to determine a correlation between blood concentration of lipids and the content of lipids in the liver of dairy cows in the transitional period.

MATERIALS AND METHODS

Late pregnant and calved cows (n = 40) were chosen from a Holstein dairy herd and divided into four groups: the first group (A) included late pregnant cows (n = 10) from the 10th to 4th day before calving; the second group (B) included late pregnant cows (n = 10) from the 4th to 1st day before calving; the third group (C) included clinically healthy puerperal cows (n = 10), whereas the fourth group (D) included ketotic cows. (n = 10). The liver and blood samples were taken from all the cows. The late pregnant cows were selected during a certain period on the basis of the time of artificial insemination and after detection of conception. Calved cows were selected as single selection in calving stalls. The diagnosis of ketosis was based on the clinical symptoms (reduced appetite, rumen atony, behavioural changes) and determined high

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concentrations of blood BHB (more than 1.2 mmol/l). Healthy cows before and after calving did not show clinical symptoms of ketosis. The experimental cows were kept in tie-up stalls in barn housing. The meal was prepared in a manner to suit the energy needs of animals in late pregnancy and early lactation. The blood samples were collected at 10:00 h or 4 to 6 hours after milking and feeding, by puncture of the jugular vein into sterile disposable test tubes without anticoagulant. After clotting for 3 hours at 4°C and centrifugation (1500g, 10 minutes, 4°C), sera were carefully harvested and stored at -20°C until analysis. Blood samples collected on fluoride were immediately centrifuged according to the same modalities and plasmas were assessed for glucose concentrations. The circulating concentrations of glucose, beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), total cholesterol (T.chol), triglycerides (TG), were determined by photometric methods using an automatic analyser Cobas Mira and the corresponding commercial kits. Shortly after blood collection, the liver was sampled through liver percutaneous biopsy using a biopsy instrument according to the Gaal's method (19) modified by Hajavcova and Kacafirek (20). The biopsy was performed at the right 11th intercostal region, approximately 2 cm below the horizontal line through the tuber coxae, with 3-5 cm long and 3-4 mm wide liver specimens. Liver tissues were histopathologically analysed for lipid contents at the Pathological Department of the Faculty of Veterinary Medicine in Belgrade. Liver specimens were fixed in neutral 10% formaldehyde solution and routinely processed. Sections obtained using a freezing microtome (Leica 1850, Jung Tissue Freezing Medium), were specifically stained with Sudan III. The liver lipid contents were semi-quantified through computer image analysis (Software Q Win) made on the appliance (Leica Q 500 MC).
The statistical analysis of the obtained data was carried out by ANOVA-procedure (Microsoft STATISTICA, ver.5.0, Stat.Soft.Inc.1995). The analysis of variance and LSD test were used to evaluate the probability of the significance of the statistical differences between mean parameter values in each group and the Pearson test was performed for evidencing significant correlations. Differences were considered as significant when p values were below 0.05 or 0.01.

RESULTS

The blood biochemistry and content of lipids in the liver performed in cows during the transition period was summarized in Table 1. As shown in Table 1, the mild liver fatty infiltration observed in healthy cows during the late pregnancy end puerperium, whereas in ketotic cows were determined moderate liver lipidosis (32.91±13.23% of lipids) and these liver lipid contents were significantly higher ($p<0.01$) than in healthy cows during transition period. The serum concentrations of NEFA and BHB were significantly higher ($p<0.01$) in the ketotic cows compared to healthy ones, whereas the mean TG a concentration was significantly lowered ($p < 0.01$) and the cholesterolemia was not significantly ($p > 0.05$) altered although this parameter was slightly decreased in the puerperal ketotic cows. In addition, the glucose concentrations was markedly depressed in the ketotic group of cows ($p < 0.01$).
The intensity of the liver fatty infiltration correlated positively and significantly ($p < 0.05$) with the markers of lipomobilisation (NEFA or BHB concentrations), but negatively and significantly ($p < 0.05$) with the circulating concentrations of compounds synthesised in liver (glucose, TG and TChol.). In the same way, the serum NEFA and BHB concentrations were positively coupled together whereas the NEFA and BHB concentrations were negatively correlated with glycaemia and the TG (Table 2).

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Table 1. Blood biochemistry and lipid contents in liver in transitional dairy cows in late pregnancy (from day 10th to day 4th before parturition, (group A); from the 4th to1st day before calving, (group B) and in early lactation (clinically healthy puerperal healthy cows, (group C); and ketotic cows, (group D) (n = 10 in each group). Results are expressed as mean ± standrad deviation.

	Group A	Group B	Group C	Group D
Liver lipids(%)	5.30 ±1.10^{A}	6.31± 1.18^{A}	8.37± 1.24^{A}	32.91± 13.23^{B}
NEFA(mmol/l)	0.27± 0.14^{A}	0.54± 0.26^{B}	0.56± 0.10^{B}	0.92± 0.12^{C}
BHB (mmol/l)	0.32± 0.14^{a}	0.41± 0.18^{b}	1.02± 0.35^{C}	2.42± 0.75^{D}
Glucose(mmol/l)	2.94± 0.32^{A}	3.12± 0.42^{A}	2.71± 0.35^{A}	1.80± 0.43^{B}
TG (mmol/l)	0.32± 0.11^{A}	0.21± 0.08^{A}	0.17± 0.06^{A}	0.12± 0.03^{B}
TChol. (mmol/l)	1.65± 0.40^{a}	1.61± 0.30^{a}	1.76± 0.62^{a}	1.39± 0.29^{a}

Legend:Values marked by letters (a, b, c, d) in one row describe significant differences; values marked by small letter differ significantly ($p < 0.05$); values marked by capital letter differ a high significance ($p < 0.01$).

Table 2. Correlation coefficients between biochemical indicators in blood and the content of lipids in liver, calculated for all groups of cows.

	NEFA	BHB	glucose	TG	T.Chol.
Fatty liver	r = 0.67**	r = 0.55**	r = -0.45 **	r = -0.55 **	r = -0.39 **
NEFA		r = 0.57 **	r = -0.52 **	r = -0.63 **	r = -0.20
BHB			r = -0.50 **	r = -0.55 **	r = -0.15
glucose				r = 0.44 **	r = 0.12
TG					r = 0.47 **

Significant correlations (*p< 0.05 or ** p< 0.01) are with asterix.

DISCUSSION

In dairy cows, it was observed that up to 50% of females exhibited some lipid accumulation in liver in the first 4 weeks after calving and that fatty liver occurs primarily in this period (8,16,17). In agreement with that, the mean liver lipid content in late pregnant and puerperal healthy cows was within the physiological range (around 5 %) but the lipid content in the liver was significantly increased ($p < 0.01$) (moderate fatty liver) in puerperal ketotic cows selected in this study. Similar results were obtained by other authors (7,8,17,14,18).

In general, circulating NEFA levels of 0.9 mmol or greater are considered to place the cow at a high risk of developing clinical ketosis and fatty liver disease. As fatty liver disease and ketosis are closely related, early detection of elevated ketone body levels can give an indication of herd risk levels. Beta-hydroxybutyrate (BHB) is commonly measured in blood or milk to assess the risk of ketosis. Levels of BHB in excess of 2.5 mmol are considered to place the cow at a high risk of suffering from fatty liver disease (8,3,18).

In the same way, the blood concentration of NEFA, considered as the best indicator of negative energy balance and of the lipomobilisation intensity during the pospartal period (16,10,6), was also significantly increased ($p < 0.01$) in the group of ketotic cows in early lactation compared to the other groups of cows. However, the NEFA concentrations were significantly increasing ($p < 0.01$) among the groups cows before calving, which showed that lipid mobilisation intensive begining from four days to one day before calving. Similar results were obtained by Veenhuizen et al. (7) and Djokovic et al. (17). In such situations, the serum BHB concentration is another indicator of energy metabolism disruptions which is more sensitive than glycaemia and which fluctuates in parallel to lipomobilisation (8,4,5,10). In the present study, ketotic cows exhibited significantly higher ($p < 0.01$) BHB concentrations than the healthy transitional cows, suggesting a strong mobilisation of fat stores.

Additionally, blood BHB and NEFA concentrations were found highly and positively correlated together ($p < 0.05$) in the current study and these 2 parameters were also significantly and positively associated ($p < 0.05$) with the liver steatosis intensity. The simultaneous and parallel variations observed between the extent of the fat infiltration in liver and the serum BHB and NEFA concentrations in puerperal ketotic cows clearly indicated that the intense lipomobilisation in the post-partum period has induced the fatty liver in the cows (16,10,11,6).

In the present study, glycaemia as indicator of energy metabolism was significantly ($p < 0.01$)

depressed (hypoglycaemia) in ketotic cows compared to healthy ones. This decrease in the glucose concentrations previously reported in different studies (6,7,15) may be related to the sudden activity of the mammary gland and the increased lactose synthesis. Furthermore, the negative energy balance associated with lipomobilisation and increased fat accumulation in hepatocytes may induce a considerable reduction in the liver gluconeogenesis, also contributing to reduce glycaemia (8,9).

On the other hand, it was observed significant decreases ($p < 0.01$) in the serum TG concentrations in puerperal ketotic cows compared to the healthy females and other biochemical parameter, at least partially synthesised in the liver, such as TChol. was also decreased ($p > 0.05$), although not significantly, during the post-partum period. In addition, these biochemical parameters positively correlated ($p < 0.05$) together, but were negatively correlated ($p < 0.05$) with the BHB or the FFA concentrations and with the liver steatosis intensity. These results suggested an increased accumulation of TG and T.chol. in hepatocytes in the puerperal ketotic cows, probably linked to a depleted liver synthesis of VLDLs as previously evoked (7,12,16,9).

CONCLUSION

This investigation demonstrated that in ketotic cows were occured a moderate degree of fatty liver (ketosis type II), whereas in healthy transitional cows were a mild fatty infiltration in liver. In parallel, the lipomobilisation markers, such as the serum BHB and NEFA concentrations, were markedly enhanced. The liver steatosis has compromised the hepatocyte syntheses leading to significantly weaker circulating concentrations of glucose and TG. All these biochemical parameters may be important biochemical indicators in determining the ketosis and fatty liver in dairy cows during transition period.

REFERENCE

1. *Overton T.R., Waldron M.R. Nutritional management of transition dairy cows: Strategies to optimize metabolic health. Journal of Dairy Science 2004, 87: E105-E119.*
2. *Dann H.M., Morin D.E., Murphy M.R., Bollerog A., Drackely J.K. Prepartum intake, postpartum induction of ketosis, and periparturient disorders affect the metabolic status of dairy cows. Journal of Dairy Science 2005, 88: 3249-3264.*
3. *Mulligan F.J., Doherty M.L. Production diseases of the transition cow. Thailand Veterinary Journal 2008, 176:3-9.*
4. *Oetzel G.R. Monitoring and testing dairy herds for metabolic disease. Veterinary Clinics of North America: Food Animal Practice 2004, 20: 651-674.*
5. *Oezel G.R. Herd level ketosis- Diagnosis and risk factors. Preconference Seminar 7C: Dairy Herd Problem Investigation Strategies: Transition Cow Troubleshooting, American association of bovine practitioners 40th Annual Conference, pp. 67-91, September 19, Vancouver, BC, Canada, 2007.*
6. *Delic B., Belic B., Cincovic M.R., Djokovic R., Lakic, I. Metabolic adaptation in first week after calving and early prediction of ketosis type I and II in dairy cows. Large Animal Review 2020, 26: 51–55.*
7. *Veenhuizen J.J., Drackley J.K., Richard M.J., Sanderson T.P., Miller L.D., Joung J.W. Metabolic changesin blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. Journal of Dairy Science 1991, 74: 4238-4253.*
8. *Jorritsma R.H., Jorritsma Y.H., Schukken P.C., Bartlett T., Wensing T., Wenting G. Prevalence and indicators of postpartum fatty infiltration of the liver in nine commercial dairy herds in the Netherlands. Livestock Production Science 2001, 68: 53-60.*
9. *Drackley J.K., Dann H.M., Douglas G.N., Janovick-Gurtzky N.A., Lutherland N.B., Underwood J.P., Loor J.J. Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. Italian Journal of Animal Science 2005, 4: 323-344.*
10. *Gonzales F.D., Muino R., Pereira V., Campos R. Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. Journal of Veterinary Science 2011, 12: 251-255.*
11. *Civelek T., Aydi I., Cingi, C.C., Yilmaz O., Kabu M. Serum non-esterified fatty acids and beta-hydroxybutyratein dairy cows with retained placenta. Pakistan Veterinary Journal 2011, 31(4): 341-344.*
12. *Sevinc M., Basoglu A., Guzulbekta H. Lipid and lipoprotein levels in dairy cows with fatty liver. Turkish Journal of Veterinary and Animal Science 2003, 27: 295-299.*
13. *Lubojacka V., Pechova A., Dvorak R., Drastich P., Kummer V., Poul J. Liver steatosis following supplementation with fat in dairy cows diets. Acta Veterinaria Brno 2005, 74: 217-224.*

ISBN 978-86-7520-611-8

14. *Djoković R., Ilić Z., Kurćubić V., Petrović M., Dosković V. Functional and morphological state of the liver in Simmental dairy cows during transitional period. Revue de Médecine Vétérinaire 2011, 162: 574-579.*
15. *Marinković M.D., Belić B. Cincović M.R., Đoković R., Lakić I., Stojanac N., Stevančević O., Devečerski G. Relationship between insulin, glucose, non-esterified fatty acid and indices of insulin resistance in obese cows during the dry period and earlylactation. Acta Veterinaria Brno 2019, 88: 143–155.*
16. *Bobe G., Young J.W., Beitz D.C. Pathology, etiology, prevention, treatment of fatty liver in dairy cows. Journal of . Dairy Science 2004, 87: 3105-3124.*
17. *Djokovic R., Šamanc H., Jovanovic M., Nikolic Z. Blood concentrations of thyroid hormones and lipids in the liver in dairy cows in transitional period. Acta Veterinaria Brno 2007, 76: 525-532.*
18. *Shen Y., Chen L., Yang W., Wang W. Exploration of serum sensitive biomarkers of fatty liver in dairy cows. Science Report 2018, 8:13574-13571.*
19. *Gaal T. Fatty liver syndrome in milch cows. Veterinarski Glasnik 1993, 47: 311-317 (In Serbian).*
20. *Hojovcova M., Kacifirek A. Liver biopsy in cattle. Acta Universiry of Agriculture Brno 1967, 36, 513-520.*

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Review
Pregledni rad

POVEZANOST METABOLIZMA GVOŽĐA, ORGANSKIH MATERIJA I FLEBOTOMIJE SA ERITROPEZOM PREŽIVARA

THE RELATIONSHIP OF THE METABOLISM OF IRON, ORGANIC MATTER AND PHLEBOTOMY WITH THE ERYTHROPOIESIS OF RUMINANTS

Siniša Grubač[a*], Marko Cincović[b], Jože Starič[c], Maja Došenović Marinković[d], Biljana Delić Vujanović[d], Jasna Prodanov Radulović[a]

[a]Naučni institute za veterinarstvo Novi Sad, Srbija
[b]Departman za veterinarsku medicinu – Laboratorija za patološku fiziologiju, Poljoprivredni fakultet Novi Sad, Univerzitet u Novom Sadu, Srbija
[c]Veterinarski fakultet Ljubljana, Slovenija
[d]Akademija strukovnih studija Šabac, Srbija
*Autor za korespondenciju: sinisagrubac@yahoo.com

S.G. https://orcid.org/0000-0002-8967-1358, M.C. https://orcid.org/0000-0002-5683-8983, J.S. https://orcid.org/0000-0002-5156-5277, M.D.M. https://orcid.org/0009-0007-8904-0197, J.P.R. https://orcid.org/0000-0002-6116-3271

SAŽETAK

Eritropeza je process stvaranja crvenih krvnih zrnaca i ona je povezana sa brojnim faktorima u organizmu. Gvožđe je značajno zbog njegove uloge u procesu stvaranja hemoglobina. Pored navedenog gvožđe je indirektni pokazatelj inflamacije i regulisano je na sistemskom i celularnom nivou, pa njegov nedostatak govori o sveukupnom zdravstvenom statusu jedinki. Deficit Fe u organizmu se odvija kroz tri faze. Najpre, u prvoj fazi, dolazi do pražnjenja tkivnih depoa, ali raste njegova ukupna količina u crkulaciji, potom sledi druga faza ili faza pravog deficita sa opadanjem koncentracije serumskog gvožđa i hemoglobina i treća faza je faza u kojoj se klinički vide znaci deficita gvožđa. Deficit gvožđa remeti sve aspekte eritropoeze. Dakle, prvo se troše rezerve gvožđa, potom sa opadanjem gvožđa koje se transportuje i obavlja funkciju menja se eritropoeza, a kada se dostupnost ovog gvožđa sasvim smanji desiće se anemija usled deficita gvožđa. Veoma veliku ulogu igra i metabolizam lipida u funkcionisanju hematopoeznih matičnih ćelija. Oksidacija masnih kiselina predstavlja glavni katabolički put kojim se proizvodi energija u matičnim hematopoeznim ćelijama. Dugolančane masne kiseline se aktiviraju u citosoli pa se transportnim sistemom transportuju u mitohondrije. U njima se dešava beta oksidacija kroz više poznatih etapa stvarajući acetil koenzim A koji pokreće ciklus trikarboksilnih kiselina. Brisanje gena za regulaciju oksidacije masnih kiselina dovodi matične ćelije hematopoeze do gubitka potencijala da se rekonstruišu i održe. Zbog značaja lipolize kod preživara i činjenice da se matične ćelije nalaze u nišama koštane srži koje su bogate lipidima, razmotrićemo i vezu između adipocita koštane srži i hematopoeze. Hronična flebotomija kod ovnova ili nedostatak Fe usled inflamacije i masne jetre kod krava dovode do specifičnih promena u crvenoj krvnoj lozi i metabolizmu organskih materija. Sve navedeno pokazuje da je potrebno poznavati metaboličke tokove kako bi se bolje shvatila eritropoeza kod preživara.

Ključne reči: preživari, eritropoeza, flebotomija, peripartalni period, metabolizam.

ABSTRACT

Erythropesis is the process of making red blood cells and it is related to numerous factors in the body. Iron is important because of its role in the process of making hemoglobin. In addition to the mentioned iron, it is an indirect indicator of inflammation and is regulated at the systemic and cellular level, so its lack speaks of the overall health status of individuals. Fe deficiency in the body takes place through three phases. In the first phase, there is emptying of tissue depots, but its total amount in the circulation increases, then follows the

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second phase or the phase of real deficit with decreasing concentration of serum iron and hemoglobin, and the third phase is the phase in which the significance of iron deficit is clinically seen. Iron deficiency disrupts all aspects of erythropoiesis. Therefore, first the iron reserves are used up, then with the decrease of transported iron, erythropoiesis changes, and when the availability of this iron is completely reduced, anemia will occur due to iron deficiency. Lipid metabolism also plays a very important role in the functioning of hematopoietic stem cells. Fatty acid oxidation is the main catabolic pathway by which energy is produced in hematopoietic stem cells. Long-chain fatty acids are activated in the cytosol and transported to the mitochondria by the transport system. In them, beta oxidation takes place through several known stages, creating acetyl coenzyme A, which starts the cycle of tricarboxylic acids. Deletion of the gene for regulation of fatty acid oxidation causes hematopoiesis stem cells to lose their potential to reconstruct and maintain themselves. Due to the importance of lipolysis in ruminants and the fact that stem cells are found in the lipid-rich niches of bone marrow, we will also consider the relationship between bone marrow adipocytes and hematopoiesis. Chronic phlebotomy in rams or Fe deficiency due to inflammation and fatty liver in cows lead to specific changes in red blood cell and blood metabolites. All of the above shows that it is necessary to know the metabolic flows in order to better understand erythropoiesis in ruminants.

Key words: ruminants, erythropoiesis, phlebotomy, periparturient period, metabolism.

METABOLIZAM ORGANSKIH I NEORGANSKIH MATERIJA I NJIHOV UTICAJ NA HEMATOPOEZU

Metabolizam organskih i neorganskih materija kod preživara pokazuje određene specifičnosti jer se radi o životinjama sa višekomornim želucem. Višekomorni želudac i kombinacija mikrobiološkog varenja u buragu i enzimatskog varenja u ostalim digestivnim partijama daje prednost kroz korišćenje biljne hrane, koja nije svarljiva za monogastrične životinje.

Gvožđe i druge mineralne materije imaju veliku ulogu u procesu hematopoeze (1,2). Gvožđe je značajno pre svega zbog njegove uloge u eritropoezi i procesu stvaranja hemoglobina. Pored navedenog gvožđe je indirektni pokazatelj inflamacije i regulisano je na sistemskom i celularnom nivou, pa njegov nedostatak govori o sveukupnom zdravstvenom statusu jedinki. Gvožđe kao mikro odnosno oligoelement ima glavnu ulogu u procesu transporta kiseonika, ali je i kofaktor mnogih drugih proteina. On prelaskom iz fero u feri oblik. Gvožđe može biti veoma toksično zbog njegove sposobnosti da veoma brzo razmenjuje elektrone, što dovodi do oksidativnog stresa pa i oksidativnog oštećenja organizma Da bi se ova njegova osobina a samim tim i toksičnost držala pod kontrolom gvožđe se transportuje vezano za proteine, a i skladišti se kao proteinski kompleks. Veoma mala količina gvožđa na nivou 0,1% se nalazi slobodno u cirkulaciji. Više od četri petina gvožđa se koristi za eritropoezu od strane eritroblasta, dok se preostala petina nalazi u mišićnom tkivu, imunološkim ćelijama i funkcionalnim enzimima. Gvožđe je veoma značajno u obezbeđivanju energije, jer učestvuje u stvaranju ATP u respiratornom lancu mitohondrija. Gvožđe ima veliku ulogu u procesu ćelijske deobe i rasta, kao i u procesu sinteze DNK.

Metabolizam gvožđa ima svoje specifičnosti zbog njegove velike reaktivnosti (3-6). Gvožđe se apsorbuje kao hemo gvožđe ili ne-hem gvožđe. Posle apsorpcije gvožđe u cirkulaciji može biti slobodno ili vezano za transferin. Mehanizam prenosa slobodnog gvožđa se i dalje izučava i smatra se da je ono prisutno u formi Fe-citrata ili Fe-acetata. Transferin je protein iz grupe beta 1 globulina i u cirkulaciji ga ima kao transferina bez vezanog gvožđa (apotransferin), transferin sa vezanim jednim atomom gvožđa (monotransferin) i transferin koji vezuje dva atoam gvožđa (di- ili holo-transferin). Fina ravnoteža između ove tri grupe proteina omogućuje da se spreče toksični efekti gvožđa. Da bi se gvožđe koristilo dalje u organizmu, potrebno je da se transferin zakači na transferinske receptore na površini ćelija. Taj receptor (TFR-1 receptor) preuzima transferin-gvožđe kompleks koji ulazi endocitozom u ćeliju, da bi se potom gvožđe oslobodilo, a transferin zajedno sa receptorom biva vraćen na površinu ćelije i otpušten u cirkulaciju da dalje obavlja svoju ulogu u transportu gvožđa. Kada gvožđe izađe iz endozoma ono može biti uskladišteno u sastavu proteina feritina, može da se koriste za metaboličke potrebe kao što je recimo sinteza hema ili može da izađe iz

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ćelije preko transporta sa feroportinom 1. Skladištenje gvožđa u ćelijama se vrši najviše u jetri i ćelijama kao što su monociti i makrofagi. Skladištenje se vrši preko feritina, koji nastaje kada se gvožđe veže za apoferitin. Feroportin 1 koji služi za transport gvožđa regulisan je peptidnim hormonom koji se zove hepcidin. Hepcidin tako omogućuje da gvožđe izađe iz ćelija, pa tako i iz enterocita ili makrofaga. Regulacija gvožđa se dodatno vrši i na subćelijskom nivou , pa su tako otkriveni geni značajni za metabolizam proteina i to DMT1 gen za ekspresiju DMT1 transportera, SLC40A1 gen za ekspresiju FPN1 transportera gvožđa iz ćelije) i TFR2gen za ekspresiju proteinskog takozavnog senzora za gvožđe.

Prilikom gubitka krvi bilo da je u pitanju krvarenje ili hronična flebotomija možemo očekivati deficit gvožđa (7-11). Pored ovoga deficit se prirodno javlja kod jedinki u prvim nedeljama života, a biva potencirana kada se životinje hrane isključivo majčinim mlekom koje je po prirodi siromašno gvožđem. Kod deficita gvožđa treba reći da je ono najčešće uzrokovano velikim gubitkom, mnogo češće nego neadekvatnim unosom. Taj gubitak se najčešće vezuje za gubitak krvi, jer je kao što smo ranije naveli ekskrecija gvožđa vrlo mala i strogo je regulisana na svim nivoima. U organizmu ima dovoljno zaliha Fe koja se lako nadoknađuju usled manjih krvarenja, pa je za nastanak deficita Fe preuslov razvoj obimnijeg krvarenja. Deficit Fe u organizmu se odvija kroz tri faze. Najpre, u prvoj fazi, dolazi do pražnjenja tkivnih depoa, ali raste njegova ukupna količina u crkulaciji, potom sledi druga faza ili faza pravog deficita sa opadanjem koncentracije serumskog gvožđa i hemoglobina i treća faza je faza u kojoj se klinički vide znaci deficita gvožđa. Klinički vidljivi znaci daju sliku opšteg umora i obamrlosti jedinke zbog manjka gvožđa te izostanka adekvatne sinteze energije odnosno ATP-a. Dodatno na apatiju može uticati i povećana proizvodnja serotonina u CNS-u, jer se smanjeno stvaraju enzimi koji ga razgrađuju, a koji u svom sastavu imaju i gvožđe. Nedostatak gvožđa smanjuje i imunološku kompetenciju jedinke, jer polimorfonukleari posle endocitoze ne mogu da ubiju unešene bakterije, obzirom da im fale enzimi koji u svom sastavu imaju gvožđe. Nađena je i smanjena svarljivost hrane zbog smanjene sekrecije enzima u digestivnom traktu. Najpre troše rezerve gvožđa, potom sa opadanjem gvožđa koje se transportuje i obavlja funkciju menja se eritropoeza, a kada se dostupnost ovog gvožđa sasvim smanji desiće se anemija usled deficita gvožđa. U našem ogledu čije ćemo rezultate predstaviti na kraju očekujemo da se razvije nalaz koji se nalazi na nivou stadijuma dva (iron depletion), odnosno da se u određenoj meri kompromituje hematopoeza (nivo 3), a da samo kod pojedinih ovnova dođe do eventualnog razvoja anemije. Razlog je taj što planiramo hroničnu, ali kontrolisanu flebotomiju, kako ne bi poremetili generalnu dobrobit životinja. Da bismo razumeli i kako je metabolizam gvožđa povezan sa opštim metaboličkim adaptacijama koristićemo i krave u ranoj laktaciji, koje će biti u klasičnoj homeorezi, a nad kojima nećemo vršiti flebotomiju. Ozirom da se očekuju blaže promene u dijagnostici izmenjenog statusa gvožđa koristićemo osim kocentracije gvožđa i parametre kao što su TIBC – kapacitet vezivanja gvožđa za transferin, UIBC– kličina ukupnog transferina koji nije zasićen gvožđem i TS%-procenat vezujućih mesta za gvožđe na transferinu. Ovo su rutinski laboratorijski parametri u dijagnostici statusa gvožđa.

Hematopoeza i metabolizam hematopoeznih matičnih ćelija su u tesnoj vezi. Značaj energetskog metabolizma i uticaja mitohondrija opisan je u najnovijem preglednom radu Morganti-ja i saradnika iz 2022.godine (12). Matične ćelije za hematopoezu se nalaze u niši koštane srži, hipoksičnom mikrookruženju koje ih primorava da se za proizvodnju energije oslanja na anaerobnu glikolizu. Faktori koji proizvode hipoksiju (Hif-1) su veoma značajno prisutni i omogućuju da ćelije hematopoeze miruju u G0 fazi, čime se čuvaju i štede za aktivaciju onda kada su potrebne, dok sa druge strane svao izaganje kiseoniku povećava oksidativno oštećenje i potenciju matičnih ćelija za hematopoezu. Matične ćelije smanjeno uzimaju glukozu u odnosu na hematopoezne ćelije većeg nivoa diferencijacije i smatra se da je to značajan metabolički mehanizam kojim se postiče njihovo mirovanje. Njihovo metaboličko mirovanje štiti matične ćelije od preranog iscrpljivanja od diferencijacije, te omogućuje da se celoživotno organizam snadeva uobličenim elementima krvi. Hematopoezne ćelije u mirnoj fazi imaju mehanizme zaštite od oksidativnog stresa, što postižu povežanim sadržajem antioksidativnih enzima kao što je superoksid dismutaza ili faktor eritroida 2. Inhibicijom ovih faktora matične ćelije gube osobine koje imaju matične ćelije kao što su samoobnova i ispravna diferencijacija, pa se dešavaju defektne hematopoeze. Oksidativni status je u tesnoj vezi sa metabolizmom glukoze.

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Prebacivanje glukoze u pentozofosfatni put umesto u ciklus trikarboksilnih kiselina je mehanizam koji smanjuje stvaranje oksidativnog stresa u matičnim ćelijama. Metabolizam proteina takođe može uticati na hematopoezne ćelije, a u prilog navedenom govori činjenica da je koncentracija aminokiselina u koštanoj srži gotovo 100 puta veća u odnosu na koncentraciju u cirkulaciji. Tako na primer glutamin podstiče ciklus trikarboksilnih kiselina i omogućuje da ćelija izađe iz mirovanja u fazu deljenja. Pored toga glutamin ima važnu ulogu u očuvanju integriteta hematopoezne linije jer stvaranje ćelija eeritroidne loze zahteva biosintezu nukleotida zavisnih od glutamina. Druga grupa aminokiselina su aminokiseline razgranatih lanaca, koji imaju veliku regulatornu ulogu tokom profilefacije matičnih ćelija, ali ne i u njihovom mirovanju. Na ogledima kod laboratorijskih miševa je pokazano da proliferacija matičnih ćelija zavisi od jedne razgranate aminokiseline – valina. Hematopoeza može biti ograničenja dostupnožu aspartata, purina i asparagina u procesu regeneracije hematopoeznog procesa. Aminotransferaza razgranatog lanca 1 je značajna u regulaciji leukomisjkih ćelija (13-15). Veoma veliku ulogu igra i metabolizam lipida u funkcionisanju hematopoeznih matičnih ćelija. Oksidacija masnih kiselina predstavlja veoma značajan put za održavanje matičnih ćelija. Oksidacija masnih kiselina predstavlja glavni katabolički put kojim se proizvodi energija u matičnim hematopoeznim ćelijama. Dugolančane masne kiseline se aktiviraju u citosoli pa se transportnim sistemom transportuju u mitohondrije. U njima se dešava beta oksidacija kroz više poznatih etapa stvarajući acetil koenzim A koji pokreće ciklus trikarboksilnih kiselina. Brisanje gena za regulaciju oksidacije masnih kiselina dovodi matične ćelije hematopoeze do gubitka potencijala da se rekonstruišu i održe. Zbog značaja lipolize kod preživara i činjenice da se matične ćelije nalaze u nišama koštane srži koje su bogate lipidima, razmotrićemo i vezu između adipocita koštane srži i hematopoeze. Istraživanja su pokazala da je broj adipocita u koštanoj srži u negativnoj korelaciji sa brojem matičnih hematopoeznih ćelija, što je deo fiziološkog procesa starenja kostiju, jer se u nekim kostima veoma brzo koštana srž napuni adipocitima, dok se u nekim kostima hematopoeza odvija celog života pa je broj adipocita manji. Nađeno je da smanjenje adipogeneze može povećati potencijal matičnih ćelija, ali sa druge strane što je veći broj adipocita posle izlaganja zračenju veća je i proliferacija matičnih ćelija. Pokazano je da su adipociti bogati faktorima koji su od značaja za oporavak matićnih ćelija nakon izlaganja zračenju. Takođe je utvrđeno da prilikom delovanja infekcije ili inflamacije neaktivne matićne ćelije uzimaju veće količine masnih kiselina, te da im one pomažu u održavanju metaboličke plastičnosti i u prilagođavanju. Prema nekim predpostavkama adipociti su neophodni kada je potrebno brzo pokrenuti hematopoezu, ali preveliki broj adipocita otežava proces hematopoeze od nivoa matične ćelije (16-19). Oksidacima masnih kiselina je mnogo niža kod ćelija u deobi, nego kod matične ćelije u mirovanju, glikoliza je visoka i kod ćelije u mirovanju i kod ćelije u deobi, dok se mnogo više ATP-a stvara kod ćelije u ciklusu u odnosu na hematopoeznu ćeliju u Go fazi.

FLEBOTOMIJA, KRVARENJA I NJIHOVI EFEKTI NA KRVNU SLIKU I METABOLIČKI PROFIL

Flebotomija predstavlja postupak uzimanja krvi u dijagnostičke ili terapijske svrhe tako što se vrši plasiranje ugle u neku anatomski dostupnu venu. Krv ima veliki potencijal samoobnavljanja, ali postoji potreba da se standardizuje način i zapremina uzete krvi, kako se čestim flebotomija ne bi životinja uvela u hipovolemiju ili anemiju.

Krv se uzima u određenoj zapremini, a određeni su standardi za različite životinjske vrste. Radi se o onoj zapremini krvi koja se može uzeti, a koja neće izazvati određena bolesna stanja kod jedinke niti jatrogenu anemiju ili hipovolemiju. Zapremine su prikazane u tabeli i odnose se na zdrave, za vrstu uobičajene, norlamne i odrasle jedinke. Ukoliko se radi o bolesnim životinjam mlađim životinjama ili životinjama koje su podvrgnute nekom eksperimentu visoke invazivnosti ove zapremine moraju biti redukovane. Ukupna zapremina krvi u cirkulaciji je 5,5-8% telesne težine životinje. Uzimanje krvi koje ne podrazumeva dodatno praćenje i koje ne može da dovede do nekog terminalnog nepovoljnog ishoda je 10% ukupne zapremine krvi. Smatra se da prikupljanje krvi u ovoj zapremini može biti ponavljano na dve nedelje kod longotudinalnih, serijskih uzimanja, kao što se to često čini kod ovnova čija se krv koristi za biotehnološke procese (npr.kod proizvodnje mikrobioloških podloga ili kod proizvodnje krvi za istraživačke svrhe. Tako se kod ovnova može uzeti 6,6 ml/kg krvi na svake dve

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nedelje. Ukoliko se koriste ovnovi rase Bosanska pramenka koji imaju oko 60 kg, to znači da se maksimalno može uzeti 396 mL krvi u jednom vađenju u dve nedelje. Uzimanje veće zapremine preko ove mora biti pod nadzorom i preporučuje se davanje određene zapremine tečnosti radi nadoknade. Odmor između dva vađenja krvi mora biti optimalan. Ukoliko se uzme oko 1% krvi sledeće vađenje se može ponoviti već narednog dana, kod uzimanja 5-7% krvi sledeće uzimanje se može vršiti za nedelju dana, ako se uzme oko 10% krvi onda period odmora do sledećeg vađenja minimum 2 nedelje, a ako se uzme 15% zapremine cirkulišuće krvi onda odmor mora trajati minimalno mesec dana, odnosno 4 nedelje (20-23). Uzorkovanje krvi se vrši iz različitih dostupnih krvnih sudova, a kod ovnova je najpovoljnija jugularna vena. Uzimanje krvi iz ove vene je relativno jednostavno i ono omogućuje dobijanje srednje do velike zapremine krvi. Uzorci dobijeni iz vene jugularis su dobrog do odličnog kvaliteta. Uzimanje krvi iz ove vene ne zahteva anesteziološki protokol, ali su potrebne mere asepse i antisepse kako bi se dobila kvalitetna i održiva krv. Višekratno uzimanje krvi iz jugularne vene može biti izvor problema, pa je potrebno da se vena, odnosno strana menja, te da se izbegavaju višekratna uzorkovanja u kratkom vremenskom intervalu iz jugularne vene.

Prilikom kontinuiranog uzimanja krvi potrebno je vršiti monitoring životinje (24-26). Monitoring podrazumeva ispitivanje hematokrita i zapremine eritrocita, da bi se ustanovilo da li životinja ima potencijal da održava stalnost zapremine eritrocita i da svaki eritrocit ima adekvatnu zapreminu, odnosno da nema razvoja mikrocitoze zbog gubitka hemoglobina i gvožđa. Zdrave, odrasle životinje mogu u okviru 24 sata da povrate zapreminu tečnosti uzete krvi, ali je za vraćanje hematokrita na adekvatan nivo i dolazak novih eritrocita potrebno vreme, pa se mora optimizirati vreme između dva vađenja krvi. Akutni gubitak veće količine krvi neće odmah pokazati promene u crvenoj lozi ili hematokritu, jer se pokreće stresna reakcija i deluju vazokonstroktori kako bi se očuvao punjenost i turgor krvnih sudova. Zbog toga ove kontrole treba sprovesti tek po isteku 24-48 sati posle poslednjeg vađenaj krvi. Smatra se da ukoliko je hematokrit ispod 35%, a koncentracija hemoglobina manja od 100g/L ne sme se pristupiti uzimanju krvi od takve životinje. Svakako potrebno je poznavati referentne i normalne vrednosti (koje su uvek nešto šire od referentnih) za vrednosti hemoglobina i hematokrita kako bi se procenilo da li se krv može uzeti, u kojim vremenskim intervalima i kojoj pojedinačnoj i ukupnoj zapremini. Ukoliko je životinja mlada ili se radi o uzimanju veće količine krvi kada je životinja u riziku od dehidracije ili prerenalne azotemije onda se posle uzomanja zapremine krvi može dodati sterilni, izotonični fiziološki rastvor, a pored njega dobro je dati rastvor Ringer-laktat.

Koje su posledice flebotomije i gubitka krvi kod životinja? U jednom starijem ogledu ispitan je redosled karakterističnih promena progresivnog deficita gvožđa pokazan je serijskim krvarenjem normalnih dobrovoljaca i pacijenata sa policitemijom (27). Radi se o ogledu sprovedenog na ljudima. Posle puštanja krvi u perifernoj krvi su se javile promene po sledećem redosledu: a) pad koncentracije hemoglobina; b) smanjeno gvožđe u plazmi; c) retikulocitoza, povećanje MCV i MCH; d) smanjenje MCV i MCH, povećanje ukupnog proteina koji vezuje gvožđe; i e) smanjen MCHC. Karakteristične promene nedostatka gvožđa su se vratile na nivoe pre flebotomije u sledećem redosledu: a) koncentracija hemoglobina; b) ćelijski indeksi; c) serumsko gvožđe; g) protein koji vezuje gvožđe u serumu; i e) hemosiderin koštane srži, i na kraju se povećana gastrointestinalna apsorpcija gvožđa vratila u normalu. Ubrzana proizvodnja crvenih krvnih zrnaca nastavljena je kod pacijenata sa policitemijom uprkos indukciji umerenog nedostatka gvožđa. Kvalitet je žrtvovan za kvantitet, i na taj način je došlo do dublje mikrocitoze nego kod normalnih subjekata sa sličnim stepenom nedostatka gvožđa.

Gubitak krvi kod ovaca može imati različite uzroke i može dovesti do anemije. U jednom ogledu za indukciju akutne anemije, 40% zapremine krvi je uzeto kako bi se procenile kliničke, hematološke i biohemijske promene i oksidativni stres izazvan akutnim gubitkom krvi (28). Osamnaest zdravih ovaca je podvrgnuto flebotomiji da bi se uklonilo 40% zapremine krvi i procenjeno je klinički i laboratorijskim testovima za kliničke, biohemijske i varijable gasa u krvi i za procenu oksidativnog stresa pre indukcije (T0), 30 min (T30 min) i 6 (T6 h), 12 (T12 h) i 24 h (T24 h) nakon gubitka krvi. Ovce su pokazale tahikardiju od T30 min do T24 h, smanjenje hematokrita, broja eritrocita i koncentracije hemoglobina, sa nižim vrednostima na T24 h i povećanjem broja leukocita od T12 h nadalje. Došlo je do smanjenja pH krvi i pritiska

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kiseonika na T30 min, povećana koncentracija laktata i smanjen bikarbonat u krvi u ovom trenutku. Nakon gubitka krvi, došlo je do povećanja srčane frekvencije počevši od vremena T30 min koji je ostao do T24 h. Brzina disanja i temperatura nisu pokazivali promene nakon početne vrednosti. Uzimanjem krvi na T0 došlo je do smanjenja vrednosti hematokrita i broja crvenih krvnih zrnaca i hemoglobina od T30 nadalje, pri čemu su niže vrednosti zabeležene na T24 h. Gubitak krvi je prouzrokovao povećanje broja leukocita od T12 h pa nadalje. Što se tiče biohemijskih varijabli, nakon uzimanja uzoraka krvi, došlo je do smanjenja ukupnog proteina i albumina od T30 min, i ove vrednosti su ostale konstantne do 24 h. U poređenju sa početnim vremenom, došlo je do povećanja koncentracije ureje od T6 h do T24 h. Porast uree je verovatno posledica prerenalne azotemije usled uzimanja velike zapremine krvi i opadanja zapremine krvi. Nije bilo varijacija u koncentraciji kreatinina i natrijuma nakon gubitka krvi. Nivoi laktata su povećani na T30, vraćajući se na početne vrednosti u kasnijim trenucima. Koncentracija kalijuma se smanjila na T6 h, nakon čega se vrednosti nisu razlikovale od početne vrednosti. Povlačenje krvi je dovelo do smanjenja pH vrednosti krvi i koncentracije bikarbonata na T30 min, sa povećanjem u narednim vremenima. Parcijalni pritisak ugljen-dioksida se povećao 24 h nakon gubitka krvi, dok se parcijalni pritisak kiseonika smanjio za 30 min i 6 h, vraćajući se na početne vrednosti u narednim trenucima. Zasićenost kiseonikom se smanjila na T30 min, sa povećanjem ove varijable u kasnijim vremenima. Posle gubitka krvi, aktivnost redukovanog glutationa je pokazala niže vrednosti na T24 h, u poređenju sa početnom linijom. Nije bilo promena u koncentraciji malonaldehida, kao ni u SOD, GPk i aktivnosti katalaze nakon gubitka krvi. Došlo je do povećanja koncentracije ureje od T6 h do kraja studije, bez promene nivoa kreatinina. Životinje nisu pokazale promene u koncentraciji malonaldehida i aktivnosti enzima superoksid dismutaze, glutation peroksidaze i katalaze, ali je došlo do smanjenja koncentracije redukovanog glutationa na T24 h. Akutni gubitak od 40% zapremine krvi je sposoban da promoviše relevantne kliničke, hematološke, gasne i biohemijske promene u krvi, i doprineo je pojavi oksidativnog stresa sa smanjenom koncentracijom glutationa, što sugeriše da ovaj proces stvara slobodne radikale u dovoljnoj količini da se smanji dejstvo antioksidanata. Došlo je do povećanja koncentracije ureje od T6 h do kraja studije, bez promene nivoa kreatinina. Životinje nisu pokazale promene u koncentraciji malonaldehida i aktivnosti enzima superoksid dismutaze, glutation peroksidaze i katalaze, ali je došlo do smanjenja koncentracije redukovanog glutationa na T24 h. Akutni gubitak od 40% zapremine krvi je sposoban da promoviše relevantne kliničke, hematološke, gasne i biohemijske promene u krvi, i doprineo je pojavi oksidativnog stresa sa smanjenom koncentracijom glutationa, što sugeriše da ovaj proces stvara slobodne radikale u dovoljnoj količini da se smanji dejstvo antioksidanata. Došlo je do povećanja koncentracije ureje od T6 h do kraja studije, bez promene nivoa kreatinina. Životinje nisu pokazale promene u koncentraciji malonaldehida i aktivnosti enzima superoksid dismutaze, glutation peroksidaze i katalaze, ali je došlo do smanjenja koncentracije redukovanog glutationa na T24 h. Akutni gubitak od 40% zapremine krvi je sposoban da promoviše relevantne kliničke, hematološke, gasne i biohemijske promene u krvi, i doprineo je pojavi oksidativnog stresa sa smanjenom koncentracijom glutationa, što sugeriše da ovaj proces stvara slobodne radikale u dovoljnoj količini da se smanji dejstvo antioksidanata. Životinje nisu pokazale promene u koncentraciji malonaldehida i aktivnosti enzima superoksid dismutaze, glutation peroksidaze i katalaze, ali je došlo do smanjenja koncentracije redukovanog glutationa na T24 h. Akutni gubitak od 40% zapremine krvi je sposoban da promoviše relevantne kliničke, hematološke, gasne i biohemijske promene u krvi, i doprineo je pojavi oksidativnog stresa sa smanjenom koncentracijom glutationa, što sugeriše da ovaj proces stvara slobodne radikale u dovoljnoj količini da se smanji dejstvo antioksidanata. Životinje nisu pokazale promene u koncentraciji malonaldehida i aktivnosti enzima superoksid dismutaze, glutation peroksidaze i katalaze, ali je došlo do smanjenja koncentracije redukovanog glutationa na T24 h. Akutni gubitak od 40% zapremine krvi je sposoban da promoviše relevantne kliničke, hematološke, gasne i biohemijske promene u krvi, i doprineo je pojavi oksidativnog stresa sa smanjenom koncentracijom glutationa, što sugeriše da ovaj proces stvara slobodne radikale u dovoljnoj količini da se smanji dejstvo antioksidanata.

U drugoj studiji koja je vršena na kozama cilj je bio da se procene i uporede efekti krvarenja sa nešto nižim intenzitetom od 25% na fiziološke odgovore kod odraslih koza sa intaktnom slezinom i

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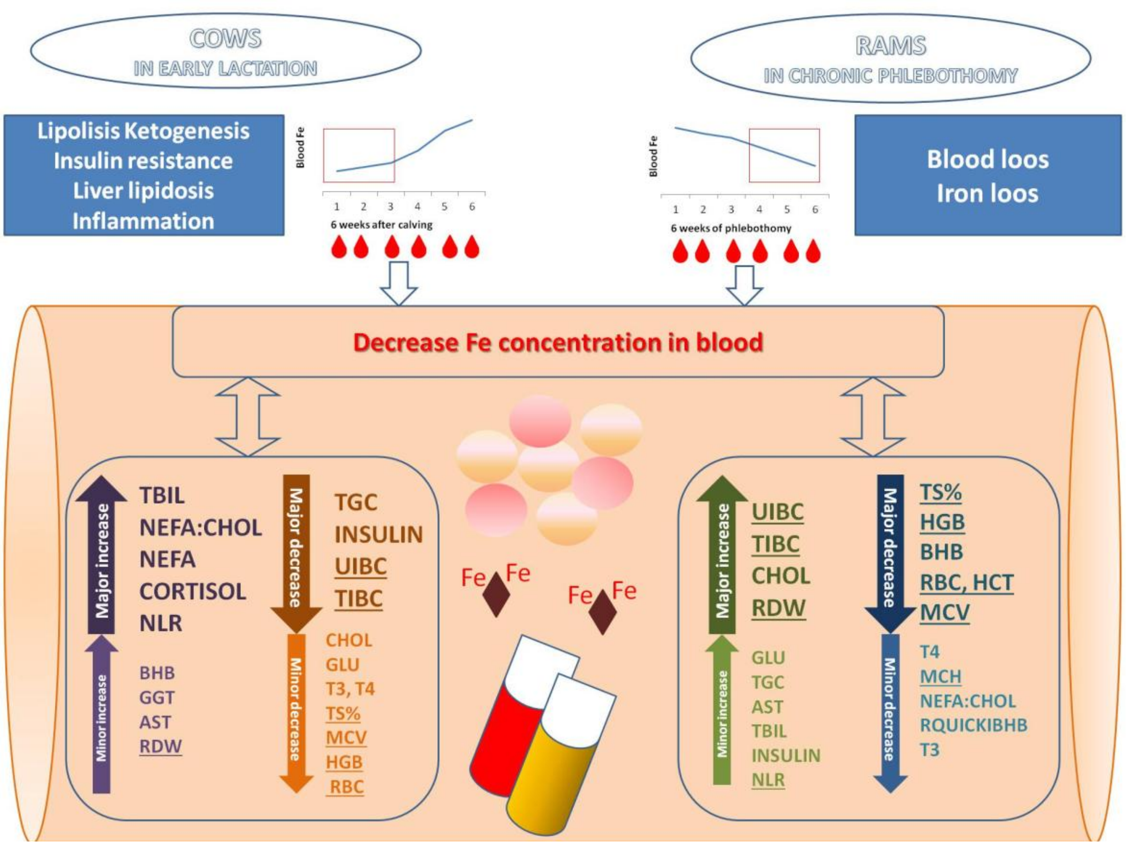
splenektomijom (29). Krvarenje je dovelo do značajnog povećanja vrednosti rektalne temperature, brzine disanja i srčane frekvencije kod normalnih i splenektomiranih životinja. Vrednosti hematokrita, koncentracije hemoglobina i ukupnog broja leukocita bile su značajno niže kod normalnih i splenektomiranih životinja sa hemoragijom. Ovi parametri su se smanjili odmah nakon krvarenja kod splenektomiranih životinja, a nešto kasnije odnosno nakon 6 sati kod normalnih životinja. Kod životinja sa hemoragijama, vrednosti limfocita, eozinofila i monocita su bili niži, dok su vrednosti neutrofila bili viši. Koncentracije ukupnog proteina i albumina u serumu su se značajno smanjile kao odgovor na krvarenje kod normalnih i splenektomiranih životinja. Nivo glukoze u plazmi se značajno povećao kod životinja sa krvarenjem. Tokom oporavka, krvarene splenektomirane životinje zadržale su više vrednosti trijasa i niže vrednosti hematokrita i hemoglobina u poređenju sa hemoragičnim životinjama koje su bile netaknute slezine.

Procena gubitka krvi je od velikog značaja u istraživačkom i praktičnom smislu. Procena gubitka krvi je od velikog značaja posebno u perioperativnom periodu i tokom hirurških operacija. Perioperativni gubitak krvi je bitan parametar u istraživanju upravljanja krvlju kod pacijenata. Međutim, trenutno ne postoji metoda „zlatnog standarda“ za njenu kvantifikaciju. Direktna merenja gubitka krvi smatraju se nepouzdanim metodama, a formule za procenu su se pokazale značajno netačnim jer su povezane sa hemokoncentracijom hemodilucijom tokom samog zahvata kao i drugim faktorima koji mogu maskirati efekte gubitka krvi. Zbog toga je u jednom ogledu utvrđeno da je procena gubitka mase hemoglobina mnogo bolji pokazatelj statusa gubitka krvi nego obračunavanje ukupne zapremine krvi. Formula podrazumeva razliku u koncentraciji hemoglobina pre zahvata i najniže postignute koncentracije hemoglobina koja je pomnožena brojem sto i telesnom masom (30). Ovo govori u prilog činjenici da hemoglobin veoma brzo reaguje posle krvarenja čak i u kontrolisanim uslovima, kao što su krvarenja toko hirurškog zahvata, a ukazuje na značaj praćenja hemoglobina i drugih parametara crvene loze kod hronične flebotomije. Pored ovoga, kod ljudi je prikazano da flebotomija čak i onda kada se uzimaju male količne krvi u cilju laboratorijske dijagnostike, ali ako se to uzimanje ponavlja bilo kroz uzimanje krvi u različite vakutajnere ili kroz ponavljanje analiza npr. u jedinicama intenzivne nege to može izazvati anemiju, pa čak postoji i posaban naziv aniemija izazvana flebotomijom (eng., *Phlebotomy-induced anemia, PIA*) (31-33). Sve navedeno ukazuje da flebotomija može biti značajan uzrok hematoloških i metaboličkih promena.

U našim ogledima smo ispitivali pocezanost koncentracije Fe sa metaboličkim, endokrinim i metaboličkim parametrima kod ovnova u modelu flebotomije i kod krava u modelu peripartalnog odgovora (34,35). Koncentracija Fe je pokazala bolju linearnu korelaciju sa metaboličkim i hematološkim parametrima u poređenju sa vrednostima TIBC, IUBC, HGB i RBC. Promena parametara krvi u funkcijama Razlog bi mogao biti taj što transferin reaguje sporije u odnosu na Fe u organizmu, zbog svog poluživota i brojnih faktora koji mogu uticati na njegovo formiranje.[218] U analizi osetljivosti „šta-ako“ ispitali smo kako bi se promenila koncentracija svih parametara da je koncentracija Fe ispod referentne vrednosti na nivou od 9 μmol /L. Nedostatak Fe kod krava je povezan sa značajnim povećanjem lipolize i ketogeneze i opterećenja hepatocita (smanjenje holesterola i triglicerida, povećanje odnosa NEFA:CHOL, TBIL i GGT), i daljim smanjenjem vrednosti TIBC i UIBC, potvrđuje da promene u Vrednosti Fe kod krava u ranoj laktaciji deo su šireg homeoretskog odgovora. Zaključeno je da se lipidi akumuliraju u jetri odraslih osoba sa nedostatkom gvožđa (36). Interesantan nalaz je da je smanjenje vrednosti gvožđa povezano sa smanjenjem vrednosti insulina. Insulin je jedini antilipolitički hormon, tako da njegovo smanjenje može da izazove lipolizu , što izaziva upalu i metabolički stres. Pokazalo se da se proizvodnja insulina može očuvati samo ako je koncentracija Fe adekvatna, pa će deficit Fe ili preopterećenje Fe narušiti ekspresiju insulina (37). Kod krava ovakve promene vrednosti Fe ne bi dovele do dramatičnih promena parametara crvenih krvnih zrnaca. Utvrđeno je da postoji razlika između funkcionalnog nedostatka Fe i anemije usled nedostatka Fe, a kod krava je nedostatak Fe očigledno funkcionalno zasnovan (38). S druge strane, kod ovnova je uočeno smanjenje BHB sa povećanjem glikemije i vrednosti insulina. Kod miševa sa nedostatkom Fe detektovano je smanjenje β-oksidacije u jetri sa povećanjem lipogeneze (39). I krave i ovnovi imaju insulinsku rezistenciju, pri čemu krave imaju problem smanjene proizvodnje insulina i nedostatak antilipolitičkog efekta, dok ovnovi imaju veću otpornost perifernog tkiva na insulin pa se i insulin i nivo glukoze povećavaju. Neka dosadašnja istraživanja su pokazala da se kod životinja sa nedostatkom gvožđa javlja mešana insulinska rezistencija, koja je u našem eksperimentu očigledno zavisila od fiziološkog statusa jedinke, odnosno da li je u pitanju jedinka u ranoj laktaciji ili mužjak (40). Kod ovnova je uočeno povećanje vrednosti holesterola i triglicerida usled povećane lipogeneze u jetri, dok su kod krava ove vrednosti smanjene usled njihove akumulacije u hepatocitima . Iako su krave i ovnovi u potpuno različitim metaboličkim stanjima, deficit Fe bi pokrenuo metaboličke procese u organizmu, koji prvenstveno utiču na metabolizam masti. Oko vremena začeća, nedostatak Fe ima tendenciju da poveća koncentraciju triglicerida u plazmi i intrahepatici, a nedostatak gvožđa može ograničiti unos masnih kiselina (41, 42). Dok su metaboličke devijacije bile dominantnije tokom nedostatka gvožđa kod krava u ranoj laktaciji, utvrđeno je da nedostatak gvožđa kod ovnova dovodi do varijacija u vrednostima crvenih krvnih zrnaca, indeksa crvenih krvnih zrnaca, kapaciteta vezivanja gvožđa i TS%, što odgovaraju promenama usled anemije i/ili funkcionalnog nedostatka gvožđa (43). Ovi rezultati potvrđuju da je deficit gvožđa kod krava u laktaciji deo šire homeorhetske adaptacije, dok je deficit izazvan hroničnom flebotomijom kod ovnova povezan sa direktnim gubitkom krvi i/ili Fe. Svi podaci i rezultati iz navedenih istraživanja sumarno su predstavljeni na Slici 1.



Slika 1. Grafički apstrakt ispitivanje hronične flebotomije ovnova i peripartalnog metabolizma kod krava na koncentraciju Fe i promena vrednosti ostalih parametara u funkciji Fe.

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LITERATURA

1. Oliveira D. C., Nogueira-Pedro A., Santos E. W., Hastreiter A., Silva G. B., Borelli P., Fock R. A. A review of select minerals influencing the haematopoietic process. Nutrition Research Reviews 2018, 31(2), 267-280.
2. Waldvogel-Abramowski S., Waeber G., Gassner C., Buser A., Frey B. M., Favrat B., Tissot J. D. Physiology of iron metabolism. Transfusion Medicine and Hemotherapy 2014, 41(3), 213-221.
3. Shayeghi M., Latunde-Dada G.O., Oakhill J.S., Laftah A.H., Takeuchi K., Halliday N., et al. Identification of an intestinal heme transporter. Cell 2005, 9;122(5):789–801.
4. 50. Sharp P.A. Intestinal iron absorption: regulation by dietary & systemic factors. Int J Vitam Nutr Res Int Z Für Vitam- Ernährungsforschung J Int Vitaminol Nutr. 2010, 80(4-5):231–42.
5. 51. Aisen P., Enns C., Wessling-Resnick M. Chemistry and biology of eukaryotic iron metabolism. Int J Biochem Cell Biol. 2001, 33(10):940–59.
6. 52. Nemeth E., Tuttle M.S., Powelson J., Vaughn M.B., Donovan A., Ward D.M., et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004, 17, 306(5704), 2090–2093.
7. Cincović M., Belić B. Opšta patološka fiziologija. Udžbenik. Poljoprivredni fakultet Novi Sad – Departman za veterinarsku medicine, 2021.
8. 54. Johns J., Heller M. Hematologic conditions of small ruminants. Veterinary Clinics: Food Animal Practice 2021, 37(1), 183-197.
9. 55. Ensley S. Evaluating mineral status in ruminant livestock. Veterinary Clinics: Food Animal Practice 2020, 36(3), 525-546.
10. 56. Radwinska J., Zarczynska K. Effects of mineral deficiency on the health of young ruminants. Journal of Elementology 2014, 19(3).
11. 57. Tvedten H. Classification and laboratory evaluation of anemia. Schalm's veterinary hematology 2022, 198-208.
12. Morganti C., Cabezas-Wallscheid N., Ito K.. Metabolic Regulation of Hematopoietic Stem Cells. Hemasphere 2022, 28, 6(7):e740. doi: 10.1097/HS9.0000000000000740.
13. Taya Y., Ota Y., Wilkinson A.C., et al. Depleting dietary valine permits nonmyeloablative mouse hematopoietic stem cell transplantation. Science, 2016, 354:1152–1155.
14. Sommerkamp P., Altamura S., Renders S., et al. Differential alternative polyadenylation landscapes mediate hematopoietic stem cell activation and regulate glutamine metabolism. Cell Stem Cell. 2020, 26, 722–738.e7.
15. Oburoglu L., Tardito S., Fritz V., et al. Glucose and glutamine metabolism regulate human hematopoietic stem cell lineage specification. Cell Stem Cell. 2014, 15, 169–184.
16. Ito K., Carracedo A., Weiss D., et al.. A PML–PPAR-δ pathway for fatty acid oxidation regulates hematopoietic stem cell maintenance. Nat Med. 2012, 18, 1350–1358.
17. Pernes G., Flynn M.C., Lancaster G.I., et al.. Fat for fuel: lipid metabolism in haematopoiesis. Clin Transl Immunology 2019, 8:e1098.
18. Schönberger K., Obier N., Romero-Mulero M.C., et al.. Multilayer omics analysis reveals a non-classical retinoic acid signaling axis that regulates hematopoietic stem cell identity. Cell Stem Cell. 2022, 29, 131–148.e10.
19. Naveiras O., Nardi V., Wenzel P.L., et al.. Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. Nature 2009, 460:259–263.
20. Hawk T.C., Leary S.T., Morris T.H. Formulary for Laboratory Animals, 3rd ed. Ames, IA Blackwell Publishing, 2005.
21. Hoff J. Methods of Blood Collection in the Mouse. Lab Animal 2000, 29(10):47-53.
22. BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement. Removal of blood from laboratory mammals and birds. Laboratory Animals, 1993, 27(1):1-22
23. Forbes N, et. al. Morbidity and mortality rates associated with serial bleeding from the superficial temporal vein in mice. Lab Animal 2010, 39(8):236-40.
24. McGuill M.W., Rowan A.N. Biological Effects of Blood Loss: Implications for Sampling Volumes and Techniques. ILAR News 1989, 31(4): 5-18.
25. Nahas K., Provost J.P., Baneux P.H., Rabemampianina Y. Effects of acute blood removal via the sublingual vein on haematological and clinical parameters in Sprague-Dawley rats. Laboratory Animals 2000, 34(4):362-71.
26. Guidelines for Blood Collection in Laboratory Animals: https://www.research.uky.edu/division-laboratory-animal-resources/guidelines-blood-collection-laboratory-animals
27. Conrad M.E., Crosby W.H. The natural history of iron deficiency induced by phlebotomy. Blood 1962, 20(2), 173-185.

28. *Sousa R.S., Sousa C.S., Oliveira F.L.C., Firmino P.R., Sousa I.K.F., Paula V.V., Caruso N.M., Ortolani E.L., Minervino A.H.H., Barrêto-Júnior, R.A. Impact of Acute Blood Loss on Clinical, Hematological, Biochemical, and Oxidative Stress Variables in Sheep. Vet. Sci. 2022, 9, 229. https://doi.org/10.3390/vetsci9050229.*
29. *Abdalla S. E., Abdelatif A. M. Physiological responses of goats (Capra hircus) to haemorrhage as influenced by splenectomy. American-Eurasian Journal of Scientific Research 2010, 5(2), 76-87.*
30. *Jaramillo S., Montane-Muntane M., Gambus P.L., Capitan D., Navarro-Ripoll R., Blasi A. Perioperative blood loss: estimation of blood volume loss or haemoglobin mass loss? Blood Transfus. 2020, 18(1), 20-29. doi: 10.2450/2019.0204-19*
31. *Bodley, T., Chan, M., Levi, O., Clarfield, L., Yip, D., et al. Patient harm associated with serial phlebotomy and blood waste in the intensive care unit: A retrospective cohort study. PLoS One 2021, 16(1), e0243782.*
32. *Chant C., Wilson G., Friedrich J.O. Anemia, transfusion, and phlebotomy practices in critically ill patients with prolonged ICU length of stay: a cohort study. Crit Care. 2006, 10(5), R140. pmid:17002795*
33. *Whitehead N.S., Williams L.O., Meleth S., Kennedy S.M., Ubaka-Blackmoore N., Geaghan S.M., et al. Interventions to prevent iatrogenic anemia: a Laboratory Medicine Best Practices systematic review. Crit Care 2019, 23(1), 278. pmid:31399052*
34. *Grubač, S., Cincović, M., Radinović, M., Potkonjak, A., Žekić, M., Starič, J., & Prodanov Radulović, J. (2024). Influence of Frequent Phlebothomy on Blood Iron Concentration, Haematological, Metabolic and Endocrine Parameters in Rams. Acta Veterinaria, 74(1).*
35. *Grubač Siniša, Cincović Marko, Djokovic Radojica, Majkić Mira, Došenović Marinković Maja, Petrović Miloš, Nikolić Sandra, Starič Jože, Prodanov Radulović Jasna. Blood iron status in dairy cows during early lactation – relationship with hematological, biochemical, endocrine and inflammatory response. Acta Scientiae Veterinariae, 2023. 51: 1933.*
36. *Ahmed U., Latham P.S., Oates P.S. Interactions between hepatic iron and lipid metabolism with possible relevance to steatohepatitis. World J Gastroenterol 2012, 18, 4651-58.*
37. *Fillebeen C., Lam N.H., Chow S., Botta A., Sweeney G., Pantopoulos K. Regulatory connections between iron and glucose metabolism. Int J Mol Sci 2020, 21. doi: 10.3390/ijms21207773*
38. *Sattar A., Mirza R.H. Haematological parameters in exotic cows during gestation and lactation under subtropical conditions. Pak Vet J 2009, 29, 129-132.*
39. *Sherman A.R. Lipogenesis in iron-deficient adult rats. Lipids 1978, 13: 473–78.*
40. *Soliman A.T., De Sanctis V., Yassin M., Soliman N. Iron deficiency anemia and glucose metabolism. Acta Biomed Atenei Parmensis 2017, 88. doi: 10.23750/abm.v88i1.6049*
41. *Davis M., Rendina E., Peterson S., Lucas E., Smith B., Clarke S. Enhanced expression of lipogenic genes may contribute to hyperglycemia and alterations in plasma lipids in response to dietary iron deficiency. Genes Nutr 2012,7, 415-425.*
42. *Georgieff M.K. Long-term brain and behavioral consequences of early iron deficiency. Nutr Rev 2011, 69: S43–S48.*
43. *Thomas D.W., Hinchliffe R.F., Briggs C., Macdougall I.C., Littlewood T., Cavill I. British Committee for Standards in Haematology. Guideline for the laboratory diagnosis of functional iron deficiency. Br J Haematol 2013, 161: 639-648.*

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UV-B LIGHT IRRADIATION DURING AUTOMATIC MILKING FOR VITAMIN D SUPPLEMENTATION IN DAIRY COWS

OZRAČIVANJE UV-B SVETLOM TOKOM AUTOMATSKE MUŽE KAO METOD ZA POBOLJŠANJE STATUSA VITAMINA D KOD MLEČNIH KRAVA

Jaka Jakob Hodnik[1,2*], Marko Jankovec[3], Jožica Ježek[1], Žiga Krusic[4], Stefan Mitterhofer[3], Jože Starič[1]

[1] Clinic for Reproduction and Large Animals-Section for Ruminants, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia
[2] Scottish Centre for Production Animal Health and Food Safety, University of Glasgow, Glasgow, United Kingdom
[3] Laboratory of Photovoltaics and Optoelectronics, Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia
[4] Bonn, Germany
* Correspondence:Jaka Jakob Hodnik 2811605H@student.gla.ac.uk

J.J.H. https://orcid.org/0000-0003-4887-0286, M.J. https://orcid.org/0000-0001-9554-7284, J.J. https://orcid.org/0000-0002-3753-5944, J.S. https://orcid.org/0000-0002-5156-5277

ABSTRACT

Vitamin D has a significant role in mineral metabolism, bone tissue homeostasis, and immunity. Cattle can synthetize vitamin D in the skin in a linear dose dependent manner of ultraviolet irradiation. The objectives of this study were to investigate the possibility of vitamin D supplementation with narrow-band UV-B irradiation during automatic milking time and to determine the influence of hair and pigmentation of skin and hair on cutaneous vitamin D_3 synthesis in dairy cows. Holstein Friesian cows from one farm were enrolled into the study. A narrow-band UV-B light (peak irradiation at 295 nm) was custom-made and mounted into the automatic milking system. Cows were split into three equal random groups using stratified sampling based on milk yield in the previous lactation, days after calving and the percentage of black area on their back. The first group was shaved and exposed to a daily dose during automatic milking, the second group was left unshaved and irradiated during the automatic milking with maximal possible dose and the third group served as control. The cows were blood sampled before and 60 days after the start of irradiation. The concentration of 25-hydroxyvitamin D was measured using an automated immune enzyme fluorescence assay. The increase in the 25-hydroxyvitamin D concentrations before and after the irradiation was statistically significant ($p>0.001$) between the shaved, unshaved and control groups. The irradiated groups shaved and unshaved produced more milk per day than the control group. Narrow-band UV-B LED light limited to automatic milking time was successful in increasing the concentrations of 25-hydroxyvitamin D in Holstein Friesian cows. Hair was reducing cutaneous vitamin D production. UV-B irradiation of cows during automatic miking is a novel way of vitamin D supplementation that does not expose farm equipment and personnel to UV-B irradiation and does not interfere with the daily routine on farm.

Keywords: cattle, ultraviolet-B light, 25-hydroxyvitamin D, milk yield, skin, hair

SAŽETAK

Vitamin D ima značajnu ulogu u mineralnom metabolizmu, homeostazi koštanog tkiva i imunitetu. Goveda mogu sintetizovati vitamin D u koži na linearni način zavisan od doze ultraljubičastog zračenja. Ciljevi ovog istraživanja bili su da se ispita mogućnost suplementacije vitamina D uz uskopojasno UV-B zračenje tokom automatskog vremena muže i da se utvrdi uticaj dlake i pigmentacije kože i dlake na kožnu sintezu vitamina

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D3 kod mlečnih krava. Holštajn frizijske krave sa jedne farme su bile uključene u studiju. Uskopojasno UV-B svetlo (vršno zračenje na 295 nm) je napravljeno po meri i montirano u automatski sistem za mužu. Krave su podeljene u tri jednake nasumične grupe korišćenjem stratifikovanog uzorkovanja na osnovu prinosa mleka u prethodnoj laktaciji, dana nakon teljenja i procenta crne površine na njihovim leđima. Prva grupa je obrijana i izložena dnevnoj dozi tokom automatske muže, druga grupa je ostavljena neobrijana i ozračena tokom automatske muže maksimalnom mogućom dozom, a treća grupa je služila kao kontrola. Krv krava je uzeta pre i 60 dana nakon početka ozračivanja. Koncentracija 25-hidroksivitamina D je merena korišćenjem automatizovanog testa fluorescencije imunoloških enzima. Povećanje koncentracije 25-hidroksivitamina D pre i posle zračenja bilo je statistički značajno ($p>0,001$) između obrijane, neobrijane i kontrolne grupe. Ozračene grupe obrijane i neobrijane davale su više mleka dnevno od kontrolne grupe. Uskopojasno UV-B LED svetlo ograničeno na automatsko vreme muže bilo je uspešno u povećanju koncentracije 25-hidroksivitamina D kod holštajn frizijskih krava. Dlaka je smanjivala proizvodnju vitamina D u koži. UV-B zračenje krava tokom automatskog muženja je nov način dodavanja vitamina D koji ne izlaže opremu i osoblje na farmi UV-B zračenju i ne ometa dnevnu rutinu na farmi.

Ključne reči: goveda, ultraljubičasto-B svetlo, 25-hidroksivitamin D, prinos mleka, koža, dlaka

INTRODUCTION

Vitamin D is an essential fat-soluble vitamin. However, some researchers regard it as a hormone due to its function in the organism. It has a significant role in mineral metabolism, bone tissue homeostasis, and immunity (Nelson et al., 2012). Cows can acquire vitamin D in various ways, e.g. via feed, parenteral injections or through UVB irradiation from the sun or artificial lighting. Vitamin D in feed can consist of either plant/fungal ergocalciferol or animal cholecalciferol. There is currently only one approved vitamin D supplement for cattle in the European Union and that is cholecalciferol. Animals can also synthesize their own vitamin D when 7-dihydrocholesterol in the skin is irradiated with UVB light, which leads to the production of cholecalciferol. The optimal wavelength of UVB light for cholecalciferol synthesis is between 295 and 300 nm (Jakobsen et al., 2015). Despite its importance, many cattle are deficient in vitamin D due to inadequate supplementation or insufficient sun exposure. In studies conducted at the Veterinary Faculty in Ljubljana, Slovenia, on permanently housed Holstein Friesian cows and calves on a commercial dairy farm, we found that blood vitamin D status was deficient in all seasons (unpublished data). Cows also experience a decline of blood concentrations of vitamin D in early lactation (0-30 days in milk) due to higher demands of calcium for milk production and decreased feed intake before and after parturition (Nelson et al., 2016; Sorge et al., 2013). Our finding and findings of other studies suggests that more attention should be paid to vitamin D supplementation in dairy cattle.

Vitamin D can be important for the prevention of some diseases, like rickets, osteomalacia and hypocalcaemia (Lean et al. 2014). A link between vitamin D and energy metabolism in cattle was demonstrated, as it influences insulin growth factor 1 (IGF1) (Rodney et al. 2018). It can be concluded that vitamin D supplementation improves cattle welfare.

MATERIALS AND METHODS

Holstein Friesian cows from a commercial dairy farm with permanent indoor housing (zero-grazing systems) were enrolled into the study. Cows were reared in loose housing with free stalls. Automatic milking has been implemented in the farm. A narrow-band UV-B light using LED technology (peak irradiation at 295 nm) was custom-made (Figure 1) so that it irradiated just back of cows (patent pending) and mounted into the automatic milking system. The light was connected to a computer that recorded exposure of individual cows to the light irradiation.

Cows were split into three equal random groups using stratified sampling based on milk yield in the previous lactation, days after calving and the percentage of black area on their back. The first group was shaved and exposed to a daily dose of almost 100 J/m2, the second group was left unshaved and irradiated maximal daily dose (more than 3x higher than in shaved group) during the automatic milking and the third group served as

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control. All cows were housed together in the same group, during the same time, receiving the same management and ration. The cows were blood sampled at the beginning of the study and 2 months after the start of irradiation. The concentration of 25-hydroxyvitamin D was measured using an automated immune enzyme fluorescence assay, using MiniVidas automatic analyser (bioMérieux, France) according to manufacturers' instructions.

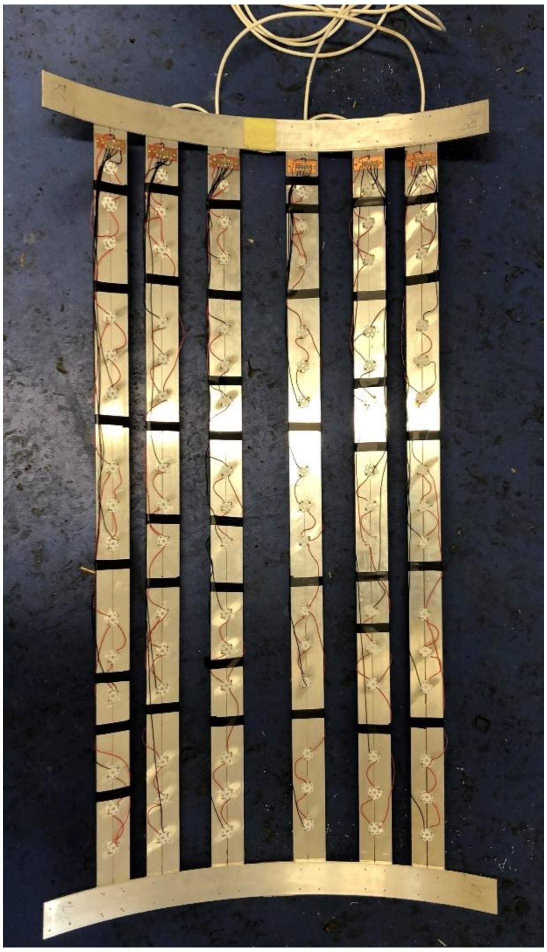

Figure 1: UV-B light that was used in the automatic milking machine

RESULTS AND DISCUSSION

The increase in the 25-hydroxyvitamin D concentrations before and after the irradiation was statistically significant ($p>0.001$) between the shaved, unshaved and control groups. The irradiated groups shaved and unshaved also produced statistically significantly more milk per day than the control group.

Use of UV-B lights during automatic milking increases blood vitamin D and could be used for supplementation of vitamin D in dairy cows that are not exposed to natural sunlight. The positive aspect of use of the UV-B light in the automatic milking machine is that it does not expose more UV sensitive skin of the cow to irradiation, as well as equipment and farm workers. In comparison to our study, a special UV light that emitted UV light with a wavelength between 250 and 400 nm was used for irradiating cows. They also showed that 25-OHD levels could be raised in blood as well as in milk with UV light exposure (Jakobsen et al., 2015). However, in our study optimal UV wavelength was used and limited only to presence in the automatic milking machine (limited by space and time), with limited skin surface of the most resilient skin exposed, which is significant advantage of our device.

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The cutaneous production of vitamin D is regulated and cannot be overdosed, which cannot be said for other ways of vitamin D supplementation. (Nelson et al., 2016; Sorge et al., 2013). Additionally with UV irradiation, vitamin D is provided to the cows independent of their daily dry matter intake.

Narrow-band UV-B LED light limited to automatic milking time was successful in increasing the concentrations of 25-hydroxyvitamin D in Holstein Friesian cows. Hair was reducing cutaneous vitamin D production. UV-B irradiation of cows during automatic miking is a novel way of vitamin D supplementation that does not expose farm equipment and personnel to UV-B irradiation and does not interfere with the daily routine on farm or with the milking process. It can be used in organic farming as well, where supplementation of vitamin D is especially costly.

LITERATURE

1. *Goff J.P. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. The Veterinary Journal 2008, 176, 50–57.*
2. *Jakobsen J., Jensen S.K., Hymøller L., Wreford Andersen E., Kaas P., Burild A., Jäpelt R.B. Short communication: Artificial ultraviolet B light exposure increases vitamin D levels in cow plasma and milk. Journal of Dairy Science 2015, 98, 6492–6498.*
3. *Martinez N., Rodney R.M., Block E., et al. Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Lactation performance and energy metabolism. Journal of Dairy Science 2018, 101, 2544–2562.*
4. *Nelson C.D., Reinhardt T.A., Lippolis J.D., Sacco R.E., Nonnecke B.J. Vitamin D signaling in the bovine immune system: a model for understanding human vitamin D requirements. Nutrients 2012, 4, 181-196.*
5. *Nelson C.D., Lippolis J.D., Reinhardt T.A., Sacco R.E., Powell J.L., Drewnoski M.E., O'Neil M., Beitz D.C., Weiss W.P. Vitamin D status of dairy cattle: Outcomes of current practices in the dairy industry. J. Dairy Sci. 2016, 99, 10150–10160. https://doi.org/10.3168/jds.2016-11727*
6. *Rodney R.M., Martinez N.P., Celi P. et al. Associations between bone and energy metabolism in cows fed diets differing in level of dietary cation-anion difference and supplemented with cholecalciferol or calcidiol. Journal of Dairy Science 2018, 101, 6581-6601.*
7. *Sorge U.S., Molitor T., Linn J., Gallaher D., Wells S.W. Cow-level association between serum 25-hydroxyvitamin D concentration and Mycobacterium avium subspecies paratuberculosis antibody seropositivity: A pilot study. Journal of Dairy Science 2013, 96, 1030–1037. https://doi.org/10.3168/jds.2012-5929*

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Article
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DETERMINATION OF NEW WELFARE AND STRESS INDICATORS ON CATTLE AND PIG FARMS BASED ON PREVIOUSLY PUBLISHED STUDIES

UTVRĐIVANJE NOVIH POKAZATELJA DOBROBITI I STRESA NA GOVEDARSKIM I SVINJARSKIM FERAMAMA NA OSNOVU RANIJE OBJAVLJENIH STUDIJA

Slavča Hristov[a], Marko Cincović[b], Branislav Stanković[a], Radojica Djoković[c], Dušica Ostojić Andrić[d], Dimitar Nakov[e]

[a]University of Belgrade - Faculty of Agriculture, Belgrade-Zemun, Republic of Serbia
[b]University of Novi Sad - Faculty of Agriculture, Novi Sad, Republic of Serbia
[c]University of Kragujevac- Faculty of Agronomy in Čačak, Čačak, Republic of Serbia
[d]Institute for Animal Husbandry, Belgrade – Zemun, Republic of Serbia
[e]Faculty of Agriculture, "Goce Delcev" University in Stip, Stip, Republic of North Macedonia
Corresponding Author: hristovs@agrif.bg.ac.rs

S.H. https://orcid.org/0000-0002-1719-9971, M.C. https://orcid.org/0000-0002-5683-8983, B.S. https://orcid.org/0000-0003-3925-6102, R.Dj. https://orcid.org/0000-0003-0900-3227, D.O.A. https://orcid.org/0000-0002-6913-4475, D.N. https://orcid.org/0000-0001-6159-5595

ABSTRACT

In recent years, numerous papers have been published that consider indicators of the welfare and stress of the animals on cattle and pig farms to improve their health and productivity. These indicators are mostly determined in numerous international projects and published in indexed journals and proceedings from international symposia. Their usefulness in on-farm assessments of animal welfare and stress is generally well recognized. In the assessments of the welfare and stress of the animals in different systems of rearing and accommodation, the need to determine new welfare and stress indicators on cattle and pig farms was observed. The paper discusses the determination of new indicators of animal welfare and stress on cattle and pig farms based on analysis of previously published studies which include the most important papers in indexed journals and proceedings from international symposia that discussed the existing indicators. The analysis of the results of those studies will be used to determine the main characteristics of the existing new welfare and stress indicators on cattle and pig farms with a focus on their applicability. The results, discussions and conclusions in those papers will be used to generate ideas to define new welfare and stress indicators of the animals on cattle and pig farms.

Key words: cattle, pig, welfare indicators, stress indicators, analyses of literature

SAŽETAK

Poslednjih godina objavljeni su brojni radovi koji razmatraju pokazatelje dobrobiti i stresa životinja na farmama goveda i svinja za poboljšanje njihovog zdravlja i produktivnosti. Ovi pokazatelji se uglavnom utvrđuju u brojnim međunarodnim projektima i objavljuju u indeksiranim časopisima i zbornicima sa međunarodnih simpozijuma. Njihova korisnost u proceni dobrobiti životinja i stresa na farmi je generalno dobro poznata. U procenama dobrobiti i stresa životinja u različitim sistemima gajenja i smeštaja uočena je potreba za utvrđivanjem novih indikatora dobrobiti i stresa na farmama goveda i svinja. U radu se razmatra određivanje novih indikatora dobrobiti životinja i stresa na farmama goveda i svinja na osnovu analize ranije objavljenih studija koje obuhvataju najvažnije radove u indeksiranim časopisima i zbornike sa međunarodnih simpozijuma na kojima se razmatraju postojeći indikatori. Analiza rezultata ovih studija biće korišćena za utvrđivanje glavnih karakteristika postojećih novih indikatora dobrobiti i stresa na farmama goveda i svinja sa fokusom na njihovu primenljivost. Rezultati, diskusije i zaključci u tim radovima biće korišćeni za generisanje ideja za definisanje novih indikatora dobrobiti i stresa životinja na farmama goveda i svinja.

Ključne reči: krave, svinje, indikatori dobrobiti, indikatori stresa, analiza literature

INTRODUCTION

Protecting health, ensuring well-being and maintaining maximum production in cattle and pigs are influenced by numerous environmental factors as well as by the status of the animal organism itself. The organism of these animals is affected by many factors from the external and internal environment of their organism that can cause stress or even distress. That is why it is very important to monitor welfare and stress through relevant indicators that will serve to reduce the negative impact of stressors from the external and internal environment of the organism. When it comes to stress animals can experience three types: physical (due to fatigue, injury, etc.), physiological (due to hunger, thirst, temperature control, etc.) and behavioural (due to the environment, unfamiliar people, surroundings, etc.). It is important to emphasize that animals might be able to tolerate a single stressor for a short time, but multiple stressors over a long period may lead to distress and suffering. The ability of cattle and pigs to cope with stress will also depend on the genetic background of the species and the animal's past experiences. Some degree of stress is inevitable during the life of animals, the aim must be to keep it to a minimum (1,2).
Animal-based indicators were mostly determined in The Welfare Quality project (3), while management-based and resource-based were considered as the Animal Need Index (4) and in Bristol Welfare Assurance Program (5). An overview of existing methods related to the aggregation of measures to produce an overall assessment of animal welfare was given by Botreau et al. (6) as well as an analysis of constraints (7).
After 2009, numerous published studies in journals and proceedings of symposia followed, which resulted in the consideration of existing and determination of new welfare and stress indicators. These studies of welfare and stress are used as a basis for the creation of main concepts, principles, indicators, parameters and criteria that are incorporated into protocols for the assessment of these very important conditions. The studies take into account the ability of animals to cope physiologically, behaviourally, cognitively and emotionally with the physicochemical and social environment (8). It should be borne in mind that assessing animal welfare and stress on cattle and pig farms is time-consuming and costly (9). There are numerous indicators of animal welfare and stress used in on-farm assessment and science, such as resource-based indicators, management-based indicators and animal-based indicators. In the scientific research that is published in indexed journals and proceedings from international symposia, new relevant indicators are continuously determined that can be used for scientific purposes and purposes of on-farm assessment.
The paper presents results, discussions and conclusions selected in published papers after 2009, intending to be used to generate ideas to define new welfare and stress indicators of the animals on cattle and pig farms, for on-farm assessment as well as for scientific research.

METHODOLOGY OF SELECTION AND ANALYSIS OF PAPERS

The authors of the paper selected in total 137 of the most significant and cited original and review papers using the Google Scholar and ScienceDirect.com database from 2009 until now in which indicators of well-being and stress in cattle and pigs are considered. After that authors identified in selected papers the most significant new welfare and stress indicators concerning the indicators identified in the Animal Need Index and Welfare Quality project. Also, when choosing indicators of welfare and stress, authors took into account the characteristics of selected indicators in terms of the possibility of use for on-farm assessment or further scientific research. Some significant papers were taken into account before 2009 because of the connection with papers after that period.

NEW WELFARE AND STRESS INDICATORS ON DAIRY CATTLE FARMS

Farm animal welfare and stress are now considered to be well-established scientific disciplines. In these disciplines, numerous multidimensional concepts and indicators have been developed based on the

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influence of factors from the external and internal environment as well as their interaction. It is well known that a wide variety of indicators should be applied to assess well-being and stress (1). The results of many studies indicate that improved cow comfort and welfare and reduced influence of stress are associated with greater farm productivity and profitability margins calculated over the replacement costs. The numerous interactions found between the management-, animal-, and resource-based welfare indicators emphasize the complexity of the association between animal well-being and farm profitability, especially in connection with the farm's management and culling strategy (10,11).

Stress is constantly present in dairy cows, especially when they are lactating. It arises as a consequence of the action of external or internal factors, i.e. external and internal stressors (12). External stressors are environmental factors such as heat or farming factors, as well as hygiene factors. The main internal stressors cause metabolic stress characterized by pronounced lipolysis, ketogenesis, insulin resistance, inflammation and immunosuppression. It arose as a result of unilateral selection for milk production and redirection of glucose to the udder, while the body uses fat for energy needs. Some stressors arise from the interaction of the external and internal environment, and these primarily cause different diseases. Stress indicators in dairy cows can be measured non-invasively (adspection, thermography, sensors, etc.), semi-invasively (routine measurement from milk during daily milking) and invasively (blood parameters). In many cases, animal-based stress response requires more specific measurements and sampling. Table 1 provides some basic information about welfare and stress indicators on dairy cattle farms that were determined in publications after 2009. In the review by de Vries et al. (13) the 27 VRHD the main types of data that are collected in national herd databases of developed countries, and related to identification and registration, management, milk production, and reproduction of dairy herds were included. Moreover, 34 WI were based on the Welfare Quality Assessment Protocol for Cattle. Twenty-three VRHDs were associated with 16 WI. The VRHD related to milk yield, culling, and reproduction were associated with the largest number of WI. Few associations were found for WI that referred to behavioural aspects of animal welfare, nonspecific disease symptoms, or resource-based indicators. For 18 WI, associations with VRHD were not significant (n = 5 WI) or no studies were found that investigated associations with VRHD (n = 13 WI). It was concluded that many VRHDs have the potential to estimate the level of animal welfare on dairy farms. As strengths of associations were not considered in this review, however, the true value of these VRHDs should be further explored. Moreover, associations found at the animal level and in an experimental setting might not appear at the farm level and in common practice and should be investigated. Cross-sectional studies using integrated welfare scores at the farm level are needed to more accurately determine the potential of VRHD to estimate levels of animal welfare on dairy farms.

In the paper by Rushen et al. (14), the automatic monitoring of behavioural indicators of animal welfare is considered. Automated feeders and devices attached to animals (e.g. accelerometers or GPS devices) can help measure the activity levels of animals with a high degree of accuracy. Technological developments have provided everyone who monitors animal welfare with a variety of tools that can be used to monitor behaviour automatically, and these have great potential to improve the ability to monitor animal welfare indicators on farms.

The authors Vasseur et al. (15) believe that a training program is needed to perform BCS accurately. Assessors need to be provided with the scoring chart and need to be trained to use this chart with proper training material.

Authors Robichaud et al. (10) in their study provide indications to farmers using freestall housing that greater cow comfort and welfare on-farm can benefit them financially through improved production. To achieve this, every aspect of housing needs to provide the highest level of comfort for the animals. Also, farmers should aim to keep lameness and leg lesions prevalence to a minimum. In this sense, the importance of good stall management in terms of dryness and cleanliness for increased milk production and quality is highlighted. The paper by Robichaud et al. (11) points out that to maximize the welfare of cows kept on tie-stall farms, every aspect of the stall needs to provide the highest level of comfort for the animals. In addition, it is indicated that minimizing the number of cows with low BCS and managing hoof health to reduce lameness also improves animal longevity and farm profitability.

Cows are animals that live in a group with a clear hierarchy, so the socialization of cows is of great importance in assessing stress or the tendency to future stress. Gibson et al. (16) have developed suitable tests that could be used to measure the sociability of individual cows on commercial farms. A standardised runway test was used as a "gold standard" test of social motivation and was repeated three times on 46 focal cows. In the runway test, the average latency to reach 5 m and 2 m from the herd and the time spent in these areas were recorded and analysed for repeatability. The results indicate that these measures could be used to assess the sociability of individual dairy cows in on-farm studies.

Body temperature during heat stress in cows, eye temperature during cow manipulation and heart rate during lameness stress were used as indicators of this response. Martello et al (17) analysed surface body temperature as an indicator of heat stress. Rectal temperature (RT), respiratory frequency (RF), body surface (BST), internal base of tail (TT), vulva (VT) and auricular temperatures (AT) were collected, from 37 Black and White Holstein cows at 0700, 1300 and 1800 hours. The AT, TT, VT and BST presented similar patterns and followed the variations of DBT throughout the day. Temperatures measured at different anatomical sites of the animal body have the potential to be used as indicative of the thermal stress in lactating dairy cows. Herbut et al. (18) concluded that THI formulas that determine the environmental risk factors for cows are unfortunately still imperfect because they take into account only factors that shape the microclimate of the air. Other indicators of cow response do not include, for example, the role of the floor (ground) in animal cooling. Since cows spend 8–16 hours a day in a lying position, at which time 20–30% of their body surface comes into contact with the ground, it will be necessary to develop a THI of the surface on which the cow is lying. Gómez et al. (19) investigated if visible eye white and eye temperature measurements are feasible non-invasive physiological indicators of acute stress in cows when they are exposed to cattle crush treatment for claw trimming. The maximum eye temperature increased during and after both situations in Brown Swiss cows, whereas in Red Holstein cows, it increased after (but not during) both situations. Kovacs et al. (20) investigated heart rate (HR) and heart rate variability (HRV) as indicators of the autonomic nervous system activity and faecal glucocorticoid concentrations as the indicator of the thehypothalamic-pituitary–adrenal axis activity in lame (with locomotion scores 4 and 5; n = 51) and non-lame (with locomotion scores 1 and 2; n = 52) Holstein-Friesian cows. HRV indices were affected by lameness. Heart rate was lower in lame cows than in non-lame ones. Vagal tone parameters were higher in lame cows than in non-lame animals, while indices of the sympathovagal balance reflected a decreased sympathetic activity in lame cows. All geometric and non-linear HRV measures were lower in lame cows compared to non-lame ones suggesting that chronic stress influenced linear and non-linear characteristics of cardiac function. Results demonstrate that HRV analysis is a reliable method in the assessment of chronic stress.

There is an increasing trend towards non-invasive cortisol measurement in cows. In research from Sharma et al. (21) cows in 54 shelters across India were assessed for historic evidence of physiological stress, through the determination of hair cortisol in 540 samples from 10 cows in each shelter by enzyme immunoassay. At a cow level, high hair cortisol concentrations were associated with dirty flanks, hock joint ulceration, carpal joint injuries, body lesions, dehydration, an empty rumen, old age, and low levels of body hair loss. Hair cortisol level promises to be an effective biomarker of stress in cows. Ebinghaus et al. (22) explored associations of faecal cortisol metabolite concentrations (FCM) with farm factors including human–animal contact, cows' fear behaviours towards humans, and milk production and udder health, involving 25 dairy farms and repeated faecal samples (n = 2625) from 674 focal cows. Farm factors via interviews and observations, avoidance distance (AD) and qualitative behaviour assessment (QBA) during a human–animal interaction were recorded. Milk yield and somatic cell scores (SCS) were calculated from milk recordings. Levels of FCMs were in general relatively low. Correlations between FCMs, QBA and SCS were significant but on a low level.

Table 1. New welfare and stress indicators on dairy cattle farms

Year	Author(s)	Some basic information about indicators in the paper
2011	De Vries et al.	The paper aims to consider the strategy to monitor animal welfare more efficiently in such a way that the level of animal welfare on a farm based on routine herd data that are available in national databases would be assessed first. It was concluded that it is not currently known which variables of routine herd data (VRHD) are associated with dairy cattle welfare indicators (WI). It was determined that twenty-three

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		VRHDs were associated with 16 WI.
2012	Rushen et al.	The authors discuss some of the issues with using automated methods to measure animal behaviour within the context of assessing animal welfare.
2013	Vasseur et al.	The author's work aims to point out the need for training assessors for a body condition score (BCS) in dairy cattle.
2019a	Robichaud	The study aims to provide indications to farmers using free-stall housing that show that cow comfort and welfare on-farm can benefit them financially through improved production.
2019b	Robichaud	The objective of this study was to evaluate the associations between the on-farm prevalence of several animal-, management-, and resource-based welfare indicators and measures of farm productivity in the use of tie-stall housing for dairy cattle.
2010	Gibbons et al.	The paper considered latency to reach the 5 m line in runway test.
2010	Martello et al.	The body surface (BST), the internal base of the tail (TT), the vulva (VT) and auricular temperatures (AT) are useful indicators of thermal stress
2012	Cincović et al.	NEFA (>0.79mmol/L) and BHB (>1.05mmol/L) are important diagnostic indicators that allow the separation of cows with parameters out of the reference values from cows with normal values of parameters in the metabolic profile and blood count.
2012	Trevisi et al.	The paper considered the Liver Functionality Index (LFI) to identify cows at risk in the transition period toward improved farm management.
2012	Alvåsen et al.	Higher mortality was associated with larger herd size, longer calving intervals, and herds that had local Holstein as the predominant breed. Lower mortality was observed in herds with a higher herd average milk yield, during the fall and winter, and in organically managed herds.
2015	Konvičná et al.	Malondialdehyde (MDA) is an indicator of oxidative stress with inverse relation with antioxidant status (ferric reducing ability of plasma (FRAP); superoxide dismutase (SOD); glutathione peroxidase (GSH-Px); selenium (Se); vitamin E in dairy cows.
2015	Kovacs et al.	Heart rate was lower in lame cows than in non-lame ones. Vagal tone parameters were higher in lame cows than in non-lame animals.
2017	Des Roches et al.	Cows were less attentive toward their surroundings (score, 0.54), had high plasma cortisol (31.3 ng/mL) and SAA (100.3 µg/mL) concentrations, and rumen temperature was increased (40.3°C).
2017	Ježek et al.	The cut-off concentration of BHB in milk set at ≥0.080 mmol/L (AUC=0.91±0.03; p<0.001) is a significant indicator for subclinical ketosis in dairy cows.
2018	Herbut et al.	Proposal: Develop a THI of the surface on which the cow is lying.
2018	Gómez et al.	The maximum eye temperature increased during and after cattle manipulation
2018	Belić et al.	Poor metabolic adaptation of cows in early lactation (eight weeks after calving) was recognized by anabolic (insulin, IGF-I) and catabolic (NEFA) indicators in the first week after calving.
2019	Sharma et al.	At a cow level, high hair cortisol concentrations were associated with dirty flanks, hock joint ulceration, carpal joint injuries, body lesions, dehydration, an empty rumen, old age, and low levels of body hair loss.
2020	Rilanto et al.	Animal-level risk factors for culling were Holstein breed, older parity, lower milk yield breeding value, older age at first calving, longer previous calving interval, having assisted calving, stillbirth and the birth of twins/triplets. Lower milk yield, somatic cell count over 200,000 cells/ml and fat/protein ratio over 1.5 at first test-milking after calving was associated with greater culling hazard during the lactation. Cows from larger herds, herds with decreasing size and higher milk yields had a higher culling probability.
2020	Ebinghaus et al.	Faecal cortisol metabolite correlates with qualitative behaviour assessment (QBA)and somatic cell scores SCS.
2020	Jerram et al.	Production values alone do not equate to high welfare and the high levels of lameness on the farm combined with its effect on salivary cortisol suggest that cow stress continues to need consideration when changing systems on commercial dairy farms.
2022	Bahrami-Yekdangi et al.	Dry period length, calf birth weight, and parity were the most important cow-level risk factors for the incidence of dystocia. Calving year, calving season, parity, twin status, dry period length, calf birth weight, calf sex, and dystocia were significantly associated with the incidence of stillbirths.
2022	Krnjaić et al.	Cows milked 3X had higher levels of NEFA, BHB, AST, GGT, TBIL, and CORT and lower levels of GLU, Ca, INS, and T4.
2022	Grelet et al.	Blood fructosamine and hair cortisol are promising indicators of chronic stress. Milk loss may be an effective and easy way to detect general problems. This may enable to monitor and reduce chronic stress in dairy farms. Heart rate was lower in the stress group.
2023	Heirbaut et al.	Milk production data (from DHI) in combination with on-farm routine measured milk fatty acid (MFA) and ketone (BHB) determined by mid-infrared (MIR), gives model DHI + BHB (MIR] + MFA (MIR] allowed to automatically predict metabolic status during early lactation.
2023	Wang et al.	Daily rumination time, daily activity, parity, body condition score, season of calving and dystocia score are indicators for ketosis included in the web application.

2023	Džermeikaitė et al.	Wearable sensors can monitor eating, rumination, rumen pH, rumen temperature, body temperature, laying behaviour, animal activity, and animal position or placement. A new farming method called "precision agriculture" and big data collection from all parts of cows' lives and production creates an opportunity for early prediction of disease.

Jerram et al. (23) investigated the long-term impact of the introduction of an automatic milking system (AMS), their study aimed to assess short-term and chronic stress associated with a change in the milking system by measuring salivary and hair cortisol levels and to assess the impact on health and production parameters. Cows from one farm changing their milking system were recruited to the study and sampled for saliva and hair before and after installation. Salivary cortisol showed no diurnal pattern but was affected by lameness and gestation. Non-lame cows showed a reduction in salivary cortisol after AMS introduction ($p < 0.001$). Hair cortisol levels increased after AMS, but it was unclear if this change was seasonal. Grelet et al. (24) compared and evaluated potential biomarkers for chronic stress after inducing stress over 4 weeks through severe overstocking, restricted access to feed and isolated unusual events. The heart rate was lower in the stress group and showed more heterogeneity at the end of the stress period. No differences were observed regarding salivary cortisol, blood glucose, β-endorphin, thyroxine and leucocyte profile. A higher level of hair cortisol and blood fructosamine was observed in the stress group at the end of the stress period. Regarding the practical use of the highlighted biomarkers, milk loss may be an effective and easy way to detect general problems, including stress. The blood fructosamine and the hair cortisol concentrations are promising indicators to assess chronic stress in commercial farms. Mortality, culling and dystocia are major stressors that most directly increase the level of stress on the farm, either through direct losses or through chronic and severe pain that the animals suffer. To predict these stressors, a large number of measurable indicators were determined to assess the occurrence of these stressors on the farm. Those factors can be classified as herd-based and cow-based and include farm size, quantity and quality of milk produced, age at first calving, period between two calvings, characteristics of calves and course of pregnancy, diseases, etc. Alvåsen et al. (25) evaluate time trends in on-farm dairy cow mortality in Sweden and identify potential herd-level risk factors. Rilanto et al. (26) identified the culling rates of Estonian dairy cows and identified the farmers' stated reasons and risk factors for culling. This observational study used registry data of all cows from herds with ≥20 cow-years in 2013–2015. Cow lactation-level analyses included data from 86,373 primiparous cows from 409 herds and 177,561 lactations of 109,295 multiparous cows from 410 herds. The most common reasons farmers stated for culling were feet/claw disorders (26.4%), udder disorders (22.6%), metabolic and digestive disorders (18.1%) and fertility problems (12.5%). Bahrami Yekdangi et al. (27) have investigated cow-level risk factors associated with dystocia and stillbirth in a relatively large sample of dairy cows using multivariable linear regression models. The incidence of dystocia was associated with the interactions of twin status × calf birth weight and twin status × stillbirth. According to our analysis, the incidence of stillbirth is caused by interactions among several factors, such as twin status × length of dry period, twin status × calving season, and twin status × parity. The highest incidence of dystocia (21.3%) and stillbirths (5.4%) was observed in hypocalcemic cows.

A particularly significant stressor in dairy cows is mastitis. Mastitis is evaluated based on the number of somatic cells in individual or pooled milk samples. Because of their pain, toxicity and systemic effect, coli mastitis is very significant, and inflammatory response indicators are also included in the evaluation of this mastitis. Des Roches et al. (28) tested behavioural and pathophysiological responses as possible signs of pain experienced by cows after an experimental intramammary challenge (mastitis) with *Escherichia coli*. Cows were less attentive toward their surroundings (score, 0.54), had high plasma cortisol (31.3 ng/mL) and SAA (100.3 μg/mL) concentrations, and rumen temperature was increased (40.3°C). In phase 3 (32 to 80 h postinoculation), bacterial concentrations decreased concomitantly with high SCC levels. Cows had high levels of haptoglobin (0.57 mg/mL) and SAA (269 μg/mL) but showed no behavioural changes. Dairy cows displayed changes in behavioural, inflammatory, and stress parameters after E. coli mammary inoculation. Our results suggest that cows may have experienced discomfort in the preclinical phase (phase 1) and pain in the acute phase (phase 2)

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but neither discomfort nor pain in the remission phase (phase 3).

Oxidative stress occurs as a result of the action of external and internal stressors and is a significant stressor in the development of various diseases. Oxidative stress was defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defences. Negative energy balance and extensive lipolysis further lead to peroxidation of released fatty acids when oxidative stress occurs. In cows, both prooxidants and antioxidants must be taken into account as indicators to gain a full insight into the level of oxidative stress in early lactation. Konvičná et al. (29) evaluated the indicators of oxidative stress (malondialdehyde, MDA) and antioxidant status (ferric reducing ability of plasma, FRAP), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), selenium (Se) and vitamin E in dairy cows. Significant changes between MDA and indicators of oxidative stress (SOD, GSH-Px, vitamin E) confirm that during parturition and onset of lactation, oxidative stress occurs in dairy cows. Exposure of peripartal cows to oxidative stress may cause an increased incidence of metabolic diseases.

A large number of extremely important diseases are metabolic diseases, and the queen of all metabolic diseases in cows is ketosis (often with fatty liver), which can be both a cause of stress and a consequence of the action of stressors. Metabolic indicators are therefore related to all other indicators of stress and well-being in dairy cows. Indicators of metabolic stress have been developed in the past 15 years in the following way: 1. the limit value of NEFA and BHB was determined, which indicates that there is a metabolically burdened and poor adaptation in cows in early lactation (30), 2. it was determined liver functional status index (LFI) which is related to adaptation and inflammatory processes in the body in cows (31), 3. cows were classified based on the values of catabolic and anabolic indicators of metabolic stress in early lactation to assess the long-term prediction of metabolic stress in cows (32). Metabolic, endocrine and immunological parameters are mostly determined from blood and are slightly invasive methods that require additional involvement of veterinarians and support staff. Therefore, in parallel with indicators in blood, indicators in milk were also determined, which would indicate metabolic stress and the health of cows. Thus, it was found that metabolic stress and subclinical ketosis in cows can be predicted based on BHB concentration in milk (33), and the introduction of mid-infrared (MIR) technology enabled the routine monitoring of metabolites from milk during the milking process (34). An increase in milking frequency is a stressogen for animals, which is measured by an increase in milk production and an increased dependence of metabolites on NEFA and BHB, which indicates an increased stress adaptation in these cows (35).

In recent times, completely new indicators or new ways to measure already known indicators of health, welfare and stress in cows have been found. These indicators are measured automatically in real-time and completely non-invasively, using different sensors. Collecting a large amount of data also results in the formation of complex linear and polynomial models within the framework of machine learning. Once determined, the models become part of easily accessible applications, in which farm-based or cow-based data can be easily entered (manually or automatically from sensors) to obtain information about the risk of developing a stressful situation such as disease. Wang et al. (36) made the XGBoost model to predict the risk of ketosis in dairy cows using machine learning models based on noninvasive prenatal indicators of parity, body condition score, dystocia score, daily rumination time, daily activity, and season of calving. In the XGBoost model, daily rumination time (60.15%) and daily activity (16.73%) were identified with the highest percentage contribution to the model, followed by parity (10.41%), body condition score (6.42%), season of calving (4.23%), and dystocia score (2.06%). The probability of ketosis increased with decreasing daily rumination time and daily activity. Moreover, parity 3+ and summer may also increase the probability of ketosis. Džermeikaitė et al. (37) reviewed the importance of the use of sensors (tail, nose, ear, leg, reticulorumen) and big data collection within the new farming method called "precision agriculture" in the early recognition of disease development as the most important stressors on the cow farm.

NEW WELFARE AND STRESS INDICATORS ON PIG FARMS

The Welfare Quality project (38) has pioneered the development of standardized methodologies and scientific instruments as protocols for the evaluation of animal welfare, providing valuable insights into the well-being of farmed animals. Despite its widespread use, many researchers have raised concerns that these protocols as time-consuming followed by a lack of score transformation that reflects in welfare outcomes and therefore costly (39). These limitations hinder its practicality for farmers. Additionally, the method used to amalgamate measures into a single welfare assessment score lacks transparency, potentially leading to debates regarding the fairness and relevance of welfare assessments based on this tool. In this context, there is a need to develop more concise methods that can effectively summarize the key aspects of the Welfare Quality® protocol (WQ) or serve as initial screening tools to identify farms with compromised welfare before implementing the complete WQ protocol. Finding new feasible indicators would therefore further contribute to the field of assessing animal welfare. Therefore, the researchers seek innovative approaches that combine behavioural and physiological indicators to advance this endeavour and further enhance the assessment of animal welfare. Such developments would contribute significantly to the field of animal welfare assessment. Table 2 provides some basic information about welfare and stress indicators on pig farms that were determined in publications after 2009.

In the most recent Scientific Opinion on the welfare of pigs on farms published by the European Food Safety Authority (EFSA) in 2022 (40), 16 significant welfare consequences were identified as highly relevant. These include restriction of movement, resting problems, group stress, isolation stress, separation stress, inability to perform exploratory or foraging behaviour, inability to express maternal behaviour, inability to perform sucking behaviour, prolonged hunger, prolonged thirst, heat stress, cold stress, locomotory disorders (including lameness), soft tissue lesions and integument damage, respiratory disorders and gastro-enteric disorders.

The fact that animal welfare at the same time is a science-based and value-based issue releases different approaches to animal welfare specification. Vitali et al. (41) introduced a novel protocol for on-farm assessing the welfare of suckling piglets. This protocol drew upon a combination of welfare parameters from existing sources, including the Welfare Quality® (38), Classyfarm (42), and AssureWell (43) protocols, either in their original form or with slight modifications. Additionally, after conducting a thorough literature review, the researchers introduced a few parameters that were not present in these existing protocols. These parameters were categorized into four groups: Qualitative Behavior Assessment (QBA), behavioural measures, lesion evaluation, and health measures. This study has shown that negative social behaviour was more frequent than positive social behaviour.

Looking for a comprehensive assessment protocol that can be used in intensive pig farming systems, Renggaman et al. (44) developed a pig welfare assessment protocol comprising 17 criteria aligned with four main principles of welfare (good feeding, good housing, good health, and appropriate behaviour). They employed a 3-point scale (0 for good welfare, 1 for moderate welfare, and 2 for poor welfare) to evaluate feeding, housing, and health, while appropriate behaviour was assessed by assessment of positive and negative social behaviours relying on qualitative behaviour assessment and human-animal relationship tests. Aggregating 25 animal-based measurements with input on 38 experts' opinions on inter-measurement and inter-stage weights Brandt et al. (45) developed an animal welfare index (AWI). Developed AWI relies on the holistic approach that combines animal, environment-, and management-based factors.

Many authors look inside environmental- and resource-based measures to find non-invasive welfare indicators. Villarroel et al. (46) develop and compare temperature and enthalpy time derivatives related to behavioural data of pigs, like latency to drink, frequency of drinking, duration of drinking and duration of resting. They concluded that times derivatives of temperature or enthalpy could be used as non-invasive welfare indicators on-farm and during transport of pigs. Stocking density, space allowance and pen size and their connection with behaviours, lesions on the body and tail, lameness scores, bursitis, body temperature, manure on the body, concentration of salivary cortisol and performance of growing pigs were used as welfare indicators in some studies (47-50). Coherently concluded that high stocking density and movement limitation reduced the welfare and performance of growing pigs. In an environment of high stocking density, pigs exhibited several notable behavioural and physical differences compared to those in the middle or low stocking density groups. Specifically, pigs in high-density environments allocated more time towards drinking and engaging in negative social interactions. Moreover, they displayed more severe body lesions, had a greater dirtiness of

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manure on their bodies, and demonstrated reduced resting times and fewer instances of positive social interactions. Conversely, pigs in the middle stocking density group exhibited a higher frequency of positive social behaviours in contrast to both the high and low stocking density groups. Elevated values of cortisol can be indicative of stress and, therefore, poor welfare (51). Vermeer & Hopster (52) established threshold values for climate-related measurements as an indication of welfare risk.

Pierozan et al. (53) established a connection between the feed conversion ratio (FCR) and daily feed intake (DFI) of growing-finishing pigs in the context of animal welfare concluding that the conditions related to poor welfare were associated with an impairment in animal performance.

During the last ten years, other novel approaches were tested to establish animal welfare indicators on farms. VHAT (Voluntary Human Approach Test) was tested by Wegner et al. (54) and HRQL (Health-Related Quality of Life) was tested by Wiseman-Orr et al. (55). Although these methods are feasible for on-farm use, they are valid only in combination with behaviour and environmental welfare indicators. Many other authors were focused on novel feasible animal-based measures as screening tools for on-farm pig welfare. Telkanranta et al. (56) used tear staining or chromodacryorrhea as a promising new indicator for pig welfare assessments. Recently, there has been growing research indicating a link between tear staining in pigs and various environmental stressors, as well as physiological markers of stress. The authors found a correlation between tear staining tail and ear damage, and approach latency. Valros et al. (57) have promising results that tail-biting lesions can be a potential measure of on-farm pig welfare, as a large range of stressors increases the risk for tail-biting outbreaks. Additionally, their research illustrates the feasibility of conducting a comprehensive tail scoring to discern various lesion types within a slaughterhouse meat inspection. In a study by Diana et al. (58), an association was established between tail, ear, and skin lesions and the production flow, revealing that all production flows were linked to a heightened risk of lesions, consequently indicating a compromise in pig welfare.

The Welfare Quality Protocol for Pigs (38) uses the Qualitative behavioural assessment (QBA) as a measure of positive emotional states through indicators of social affiliative behaviours, exploratory behaviour and play behaviour. The protocol states that negative emotions such as distress or fear should be avoided whereas positive emotions such as contentment should be promoted. Moreover, the Five Freedoms framework primarily addresses the negative aspects of animal welfare, except for the freedom to express natural behaviour. Various alternative approaches have emerged to emphasize positive animal welfare. These include the "quality of life" perspective, the positive emotions approach, the positive affective states approach, and the happiness approach (59). The "quality of life" (QoL) approach, as proposed by Rowe & Mullan (60), seeks to enhance animals' lives by providing them with pleasures, comforts, and a balanced ratio of positive to negative experiences. The positive emotions concept lies in the fact that animals are not only capable of experiencing short-term emotions triggered by events in their environment, such as pleasure, but also long-term emotions like happiness (59). Furthermore, Mellor (61) introduced the concept of positive affective states within the realm of positive welfare. This term encompasses a broader range of experiences than emotions, including all subjective feelings and sensations that animals consciously perceive as pleasant or unpleasant. These affective states motivate animals to behave in particular ways, directing their actions toward achieving specific goals, whether those attempts result in success or failure. However, a multidimensional scientific approach is needed for feasible on-farm measurement and evaluation of positive animal welfare.

Table 2. New welfare and stress indicators on pig farms

Year	Author(s)	Basic information about indicators in the paper
2010a	Faucitano et al.	Anamnestic data of feeding or fasting time and resting in lairage
2011	Villarroel et al.	Temperature time derivatives and enthalpy time derivatives related to behavioural data: latency to drink (the time the pigs waited after unloading to drink), frequency of drinking (the number of times each pig engaged in a drinking bout), duration of drinking (total amount of time spent drinking) and duration of resting (the total amount of time sitting or lying)

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2011	Nielsen	Data warehouse for assessing animal health, welfare, risk management and-communication
2015	Knage-Rasmussen et al.	Routinely collecting data along or after the production process, such as the use of routine meat inspection
2011	Wiseman-Orr et al.	Health-related quality of life (HRQL)
2014	Dokmanović et al.	Time spent in the abattoir depot significantly affected blood lactate, carcass rigour mortis, skin damage, drip loss, colour and meat quality of slaughtered pigs
2014	Valros et al.	Higher abscesses and arthritis occurrence rate, pour carcass characteristics, rise in condemnations at slaughter and pourer meat quality
2014	Vermeer et al.	The effect of stocking density and space allowance on welfare (skin and tail lesions, lameness scores) and performance parameters
2015b	Mellor	Feeding behaviour, sexual and mating behaviour
2015	Renggaman et al.	Pig welfare assessment protocol that combines animal-, environment-, and management-based measures
2016	Telkanranta et al.	Tear staining or chromodacryorrhea
2016	Fu et al.	The effect of stocking densities on welfare indicators, such as behaviours, lesions on the body and tail, body temperature, manure on the body and concentration of salivary cortisol of growing pigs
2016	Meyer-Hamme et al.	The effect of group size on various animal-based measures of the Welfare Quality® protocol for growing pigs (body condition, bursitis, manure, wounds, tail biting, lameness, laboured breathing, scouring, skin condition (inflammation or discolouration), hernias, twisted snouts and rectal prolapse)
2017	Van Staaveren et al.	Carcass tail and skin lesions at meat inspection for the assessment of pig health and welfare on farms (poor body condition, bursitis and severe tail lesions)
2017	Brown et al.	Play behaviour
2017	Spinka	Exploratory and feeding behaviour
2017	Matthews et al.	Automated system with a single type of sensor–a depth video camera–to track 3D pig positions and measure multiple behaviours non-invasively
2017	Brandt et al.	Animal Welfare Index (AWI)
2018	Ahloy-Dallaire et al.	Play behaviour
2018a; 2018b	Marcet Rius et al.	Tail and ear movement
2018	Vermeer and Hopster	Climate-related measurements
2018	Amos et al.	The Business Benchmark on Farm Animal Welfare
2019	Keeling	Exploratory behaviour, play behaviour, social affiliative behaviours, synchronization
2019	Rault	Pro-social behaviours
2019	Vigors and Lawrence	Qualitative interviews to directly examine livestock farmers' perspectives of positive welfare
2019	van Staaveren et al.	The PIG WELFare INDicators (PIGWELFIND) project (Research Stimulus Fund 11/S/107) was developed to progress the development of ante and post-mortem MI (Meat inspection) as a pig health and welfare diagnostic tool
2020	Valros et al.	Validation of a scoring system sensitivity for properly differentiating farms with different levels of tail-biting
2020	Vullo et al.	Piglet Grimace Scale (PGS), a facial-expression-based pain coding system
2020	Blomke et al.	Development and evaluation of an automated system for the assessment of ear and tail lesions as welfare indicators in pigs at the abattoir
2020	Pierozan et al.	Animal welfare indicators (Welfare Quality® assessment protocol for pigs) and their possible associations with feed conversion ratio (FCR) and daily feed intake (DFI) of growing-finishing pigs
2020	Camerlink & Ursinus	Ear and tail postures
2020	Vitali et al.	Behavioural, lesion and health measures
2020	Wegner et al.	VHAT (Voluntary Human Approach Test)
2020	Courboulay et al.	BEEP: An advisory pig welfare assessment tool developed by farmers for farmers
2020	Pfeifer et al.	"Animal Welfare Indicators: Practical Guide—Pigs" developed by the German Association for Technology and Structures in Agriculture
2020	Statham et al.	The Rapid Defence Cascade (DC) response (startle, freeze): (i) sparse feature tracking computer vision image analysis of 200Hz video, (ii) load platform, (iii) Kinect depth camera, and (iv) Kinematic data.

2020	Haigh et al.	Open Field (OF) and Novel Object (NO) tests
2020	World Animal Protection	Animal Protection Index
2020	Sandoe et al.	Welfare scores for 15 dimensions
2021	Laurijs et al.	Vocalizations
2021	Gomez et al.	Precision livestock farming (PLF) technologies: vision-based solutions, load-cells, accelerometers and microphones, thermal cameras, photoelectric sensors, radio-frequency identification (RFID) for tracking, infrared thermometers and pyrometers
2021	Hansen et al.	Grad-CAM for assessment of the Pig Grimace Scale
2021	Larsen et al.	Information technologies (sensors) for welfare monitoring
2022	Papageorgiou	The “quality of life” approach, the positive emotions approach, the positive affective states approach, and the happiness approach
2022	Rowe & Mullan	Good Life Frameworks for Pigs
2023	Andersen et al.	Associations of group size, floor space and type of feed with selected welfare indicators (proportion of pigs per pen with bite marks on ears, body and tail, hernia, and movement disorders, and proportion approaching vs. fleeing from an unfamiliar human) and performance indicators (daily weight gain, feed conversion ratio, and mean weight of the slaughtered pigs) in finishing pigs
2023	Michelsen et al.	Danish Animal Welfare Index (DAWIN)
2023	Plut et al.	Combination of Serum and Oral Fluid Cortisol Levels

Papageorgiou (62) in his master thesis makes a review of behavioural indicators for positive animal welfare. Based on literature data meta-analysis, he found out that play is the behaviour that most frequently has been studied as a positive welfare indicator. The content and neurobiological foundation of play suggests that an animal engaged in play is experiencing a positive state of well-being (63). Some studies deal with the experimental assessment of play behaviour (64-68), while others studies explain the concept of play behaviour theoretically (63, 69-71, 59). The motivation of animals to play is very strong because of the pleasure they experience leading to good welfare (69). Indeed, maternal care, social affiliative behaviours, social play, and synchronization have been discussed as potential positive welfare indicators for pigs. These behaviours are essential aspects of pigs' natural behaviour and social dynamics, making them valuable indicators when assessing the well-being of these animals. Mellor (63) directs attention toward feeding behaviour, highlighting the pleasure animals derive from exploring and savouring their food. In contrast, Keeling (70) concentrates on exploratory behaviour as a potential indicator of positive welfare, albeit with some reservations related to the possibility of fear-induced exploration. According to Keeling (70), exploration is tied to cognition and can be categorized into two types: inquisitive exploration, where animals seek change, and inspective exploration, where animals respond to changes in their environment. Keeling (70) also delved into the concept of synchronization as a potential indicator of positive welfare in pigs, recognizing their inherently social nature. Given that pigs possess a strong innate drive to explore and root, often dedicating a significant portion of their time to foraging in their natural habitats (72), both exploratory and feeding behaviours emerge as promising indicators of positive welfare for domestic pigs. Additionally, pigs form close social bonds not only with related individuals but also with unrelated ones, particularly when they have been raised together. Pro-social behaviours, for example sharing resources of space and food with other individuals in the group, can be considered a positive welfare indicator in pigs when they happen consistently (73). Based on the literature results, sexual behaviour is another animal-to-animal interactive behaviour which indicates that the individuals are experiencing positive affective states (63). Social affiliative behaviours, reflecting these strong social connections, hold the potential as positive welfare indicators. Rius et al. (74) and Marcet-Rius et al. (75) stated that tail and ear movements in pigs are promising positive welfare indicators for the on-farm welfare assessment. Nonetheless, implementing positive animal welfare measurements at the farm level can be a complex endeavour.

Vocalizations in pigs have indeed been the subject of extensive study. However, to establish vocalizations as reliable positive welfare indicators, more investigation and understanding of the nuances of pig vocalizations are required, including differentiating between types and contexts of

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vocalizations, It's worth noting that research has shown that certain vocalizations, such as screams, are generally indicative of negative emotions (76). These vocalizations are employed, for example, in the Welfare Quality protocol to measure fear through Qualitative Behavior Assessment (QBA) in slaughterhouse settings.

Pain assessment in pig farming faces practical limitations, due to the lack of reliable and feasible tools. Vullo et al. (77) and Hansen et al. (78) used the Piglet Grimace Scale (PGS) as a coding system for facial expression related to on-farm surgery interventions and making distinctions between stressed and unstressed pigs. The authors found that the PGS score increased after surgery and inter-observer reliability was excellent.

To perceive the on-farm feasibility of positive welfare indicators Vigors & Lawrence (79) developed questionnaires to investigate the beliefs and attitudes of farmers toward perspectives of positive welfare. Findings reveal that farmers describe elements of positive welfare which are broadly in line with indicators suggested in the positive welfare literature. Furthermore, this study reveals that farmers tend to prioritize the mitigation of negative welfare aspects as their foremost management concern. Positive welfare is often construed as a consequence of addressing these negative aspects, rather than being directly addressed as a separate and explicit goal in farm management.

Integrating indicators for pig health and welfare into meat inspection processes holds the potential to reduce the necessity for on-farm assessments. Notably, skin and tail lesions are significant welfare indicators in pigs, offering a promising avenue for data collection during meat inspections. These indicators may serve as indirect, "iceberg" markers of on-farm welfare conditions. As noted by Van Staaveren et al. (80), both carcass tail and skin lesions have demonstrated the capacity to account for the prevalence of various welfare issues on farms, underscoring their potential as valuable umbrella indicators in this context. These findings were results from the project PIG WELFare INDicators (PIGWELFIND) with the main aim of the development of ante- and post-mortem meat inspection as an on-farm pig health and welfare diagnostic tool.

Many authors have promising results in using Precise Livestock Farming technology for the assessment of pigs' welfare on-farm or in the abattoir. Matthews et al. (81) introduced an innovative automated system that utilizes a single type of sensor, specifically a depth video camera, for tracking 3D pig positions and non-invasively measuring various behaviours. These behaviours encompass standing, feeding, drinking, and locomotor activities. This automated system is suitable for use in commercial farms because it offers continuous monitoring of multiple behaviours, providing metrics that are not only more intuitive but also possess diagnostic validity. This technology holds promise for enhancing the welfare assessment and management of pigs in practical, real-world contexts. To enable cross-country comparisons of pig welfare, Benchmarking Farm Animal Welfare tools were defined, covering several welfare dimensions and features typically modified in legislative and market-driven welfare initiatives aimed at pig production (82-84).

Basically, on-farm welfare assessments are the main method for assessing pig welfare, but these approaches are hard to do and time-consuming (85), and they include an increased risk of biosecurity and disease transmission within and between farms (86). As is given in Table 2, it makes an increased interest in routinely collecting data along or after the production process (87,88).

The focus of several studies was to evaluate the welfare of dairy herds based on routinely collected data, including at meat inspection (89-91), but there is limited work on the use of routine meat inspection data for pig health and welfare assessment (87,88), such as incorporating welfare indicators during meat inspection at abattoirs as a surveillance tool for pig health and welfare (92-94). Friedrich et al. (95) suggested the use of so-called “iceberg” indicators to assess overall animal welfare and to provide a picture of the overall welfare of the animal and function as a warning signal for underlying problems. Tail and skin lesions are among the most frequently cited animal-based indicators of pig welfare and expert panels proposed to use them in finishing pigs (96). As is well noticed in the van Staaveren et al. (80) study, incorporating indicators for pig health and welfare at meat inspection could reduce the need for on-farm assessments. During meat inspection all body parts are available and it is very easy to notice body lesions, which leads to conclusions regarding slaughter animals' pre-mortem welfare. Van Staaveren et al. (80) used an adapted version of the Welfare Quality protocol inspecting pigs of different ages (4-8 wk, 8-13 wk and 13-23 wk). The average prevalence of welfare outcomes for each stage was calculated. One batch of pigs was observed at slaughter and skin and tail lesions were scored according to severity for each carcass. The average prevalence of carcass lesion outcomes was calculated for each farm, using linear regression models to predict the prevalence of each welfare outcome in each stage based on the prevalence of the different carcass lesions.

Van Staaveren et al. (97) made recommendations to further progress the development of meat inspection as a pig health and welfare diagnostic tool and address some of these barriers since obtained findings can act as a valuable source of information on pig health and welfare. The PIG WELFare INDicators (PIGWELFIND) project (Research Stimulus Fund 11/S/107) was developed to progress the development of *ante* and *post-mortem* meat inspection as a pig health and welfare diagnostic tool in Ireland. Investigators organized three multi-stakeholder focus groups to explore areas of conflict and agreement between stakeholders' vision for including pig health and welfare indicators in meat inspection and how to achieve this vision. Each focus group consisted of eight stakeholders: pig producers, Teagasc pig advisors, pig processors, veterinarians involved in meat inspection, private veterinary practitioners, and personnel with backgrounds in general animal health and welfare and food safety policy. In general, stakeholders expressed positive attitudes towards the use of meat inspection data to inform pig health and welfare when standardization of recording and feedback is improved, and the meat inspection system provides real-time benchmarking possibilities. Most emphasis was placed on health indicators as a priority, while it was felt that welfare-related indicators could be included after practical barriers had been addressed (i.e., line speed/feasibility, standardization and training of meat inspectors, data ownership).

The combination of physiological and behavioural indicators could provide useful information on the welfare state of an animal. Research performed by Candiani et al. (98) to identify pig welfare indicators that could help in recognizing on-farm stressful practices. The study evaluated behavioural and physiological indicators (cortisol and negative acute phase proteins) in 2 groups of 20 female pigs 4 months old after a 48-hr transport. The first group (A) was transported at the end of May, and the second (B) in June. Behavioural observations and blood collection occurred at arrival (D1) and 28 days later (D28). Compared with within-animal control samples obtained 28 days later, pigs of Group A had increased cortisol levels and decreased albumin concentrations after arrival. As demonstrated by lesion and behaviour observations, the effect on cortisol and albumin was higher in Group B pigs after a tail-biting episode occurred. The study has reported no evidence of Retinol Binding Protein (RBP) in pigs. A method developed for swine RBP quantification found RBP strongly reduced in D28 samples of Group B, confirming it to be a negative protein in pigs.

According to Valros et al. (99), tail biting is a common and serious welfare-reducing problem in pig production. The occurrence rate of slaughtered pigs in countries where tail docking is prohibited is 6-11.7% (100,101) and about 3% in countries where tail docking is allowed (101). Affected pigs are more prone to abscesses and arthritis (102,103), and have adverse effects on carcass characteristics, as it may reduce growth (103,104) and cause an increase in condemnations at slaughter (100,104). Reduced welfare increases the risk of tail biting (105,106) and can also have negative consequences on meat quality (107). Even though there are many reasons to suppose that tail biting is linked to underlying stress (108,109) and that being a victim is stressful (110), there is still scarce information available on the consequences of tail biting to the victim.

The evaluation of prolonged or repeated psychological stress is challenging, and to get a reliable picture several measures should be used (111). Cortisol is a traditional measure of stress in pigs, being elevated by acute stress (112), but the effects of chronic stress on cortisol concentrations are less straightforward (99). Studies on humans and laboratory animals show that chronic stress or pain appears to ultimately cause a reduction in daily overall cortisol secretion, as well as in cortisol reactivity to stressors (113), confirmed by similar results in pigs housed in barren environments or under repeated noise stress (114-116).

Premortem stress affects muscle *post-mortem* pH. DFD (Dark, Firm, Dry meat) occurrence is associated with long-lasting pre-slaughter stress, *e.g.* during handling, transport and slaughterhouse lairage as well as a long fasting time (117-119); the glycogen reserves are reduced already before slaughter, due to the stress-induced degradation of muscle glycogen, and the ultimate lactic acid is lower than normally resulting in a pH value higher than 6.0. In the PSE case, those pigs that still have a

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normal glycogen level at slaughter and that have experienced psychological and/or physical stress just before slaughter have a fastened muscle glycogen breakdown *perimortem*. Lactic acid accumulates in the muscle when the muscle temperature is still high, and this combination causes a partial denaturation of meat proteins and thus a light colour and softness as well as a decrease in water-holding capacity. In addition, heat shock proteins (HSPs) are a potential measure of chronic stress. Cells react to stress by synthesizing HSPs, which help them to maintain intracellular protein homeostasis. HSP-induction is caused by several different cell-level stressors (120). Among the stress-inducible HSPs, the response of HSP70 has been studied most extensively. Its synthesis peaks 8-10 hours after stress, and the concentration stays high for several days (120). Therefore, short to intermediate transport and pre-slaughter handling may not last long enough to affect the amount of HSP70 and it has been speculated to reflect stressors the pigs have encountered on the farm (121). The study by Valros et al. (99,100) also indicates that HSP70 is a promising measure for chronic stress, while the cortisol response during acute stress is not an unambiguous reflection of previously experienced stress levels. The study showed support for the fact that tail-bitten pigs might produce less lean meat per carcass.
Withholding food from pigs before transport to the abattoir from the farm and keeping pigs in lairage before slaughter is a common practice, often regulated by law (122). It varies, but recommended times are usually between 12 and 24 h (123), although some studies have described up to 36 h (124). The reasons for feed withdrawal include many factors, such as higher animal welfare during transport, transport losses and travel sickness reduction, and a possible reduction in the incidence of pale, soft and exudative (PSE) meat (123). Nevertheless, pigs for commercial purposes are subjected to times of total fast (on-farm fasting plus transport time plus lairage) for an average of 46,5 h and can remain in the lairage for less than 24 h without being fed (125). This is an aspect that can impact the quality of the meat and cause a high incidence of dark, firm, and dry (DFD) meat (126), demonstrating the usefulness of a previous fast on the farm, a short transport time with homogeneous groups and without social mixing. These aspects favour handling during loading, unloading, and positive social behaviour during lairage. Physiological indicators show that transport and pre-slaughter management represented a challenge for the animals, which was compensated with rest times in the abattoir of fewer than 8 hours, allowing the recovery of the pigs, and leading to physiological and behavioural changes induced by stress (127). Meat inspection *post-mortem* changes, such as PSE and DFD meat occurrence rate, combined with data regarding time spent in the abattoir depot and feed withdrawal times before slaughter, may be used in finished fattening pigs stress assessment.
Dokmanović et al. (128) noticed that time spent in an abattoir depot significantly affected blood lactate, carcass rigour mortis, skin damage, drip loss, colour and meat quality of slaughtered pigs. In addition, the handling procedure influenced blood lactate, pH and temperature 60 minutes after slaughter, and may be seen as potential stress indicators. Long lairage is more stressful, and is detrimental to carcass quality, but causes better meat quality compared to short lairage. Rough handling was related to higher lactate and lower meat quality.

CONCLUSSION

Based on the analysed literature data related to the determination of new welfare and stress indicators on cattle and pig farms presented in the paper from previously published studies covering the period from 2009 to today, the following can be concluded:
in the analysed period, completely new indicators or new methods to measure already known
indicators of health, welfare and stress in cows and pigs have been found. These indicators are measured automatically in real-time and completely non-invasively, using different sensors. Collecting a large amount of data also results in the formation of complex linear and polynomial models within the framework of machine learning;
cattle and pig welfare and stress could be assessed by observing or measuring many physical, physiological or behavioural features of the animals and qualities of the animal's environment;
welfare and stress are complex, so it is usually important to assess more than one indicator to reveal the extent to which welfare is good or bad, and stress is present, rather than assessing just one aspect of the animal's biology or environment; there are three main sources of welfare and stress indicators: 1. the animal in its current situation (e.g. frequencies or

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durations of abnormal behaviour, concentrations of hormones or body condition), 2. the animal in a decision-making test (e.g. preference tests and cognitive bias tests) and 3. the animal's environment or situation (e.g. quality and quantity of the diet, presence of a hiding place, exposure to weather, or details of husbandry routines, etc.);
welfare and stress indicators can be measured via a continuum between two main approaches: 1. objectively, e.g. quantifying rates, durations, frequencies, concentrations or intensities and subjectively, e.g. owner/keeper questionnaires, qualitative behaviour assessment, lameness or pain scoring systems, etc. and which welfare and stress indicators should be assessed depends partly on whether concepts of welfare and stress include the animal's feelings, physical functioning, and/or naturalness. Feelings can be crucial to some concepts of welfare and stress, e.g. even healthy animals living in a naturalistic habitat could have poor welfare if they are anxious, bored, or socially stressed. Despite feelings being private to each individual, it is possible to measure the behavioural and physical signs of those underlying experiences.

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REFERENCE

1. *Broom D.M. Broom and Fraser's Domestic Animal Behaviour and Welfare. 6th Edition. CABI, 2021.*
2. *Webster J., Margerison J. (Eds.). Management and welfare of farm animals: the UFAW farm handbook. John Wiley & Sons, 2022.*
3. *Canali, E., Keeling, L. Welfare Quality® project: from scientific research to on farm assessment of animal welfare. Italian Journal of Animal Science, 8(sup2), 900-903, 2009.*
4. *Bartussek H., Leeb C.H.M., Held S. Animal Needs Index for Cattle—ANI35L/2000—cattle. Federal Research Institute for Agriculture in Alpine Regions BAL Gumpenstein, Irdning, Austria, 2000.*
5. *Leeb C.H., Main D.C.J., Whay H.R., Webster A.J.F. Bristol Welfare Assurance Programme - Cattle assessment. University of Bristol, UK, 2004.*
6. *Botreau R., Bonde M., Butterworth A., Perny P., Bracke M.B.M., Capdeville J., Veissier I. Aggregation of measures to produce an overall assessment of animal welfare. Part 1: a review of existing methods. Animal 2007, 1(8), 1179-1187.*
7. *Botreau R., Bracke M. B. M., Perny P., Butterworth A., Capdeville J., van Reenen C.G., Veissier, I. Aggregation of measures to produce an overall assessment of animal welfare. Part 2: analysis of constraints. Animal 2007, 1(8), 1188-1197.*
8. *Veerasamy S., Lakritz J., Ezeji T., Lal R. Assessment methods and indicators of animal welfare. Asian Journal of Animal and Veterinary Advances 2011, 6(4), 301-315.*
9. *De Vries M., Engel B., Den Uijl I., Van Schaik G., Dijkstra T., de Boer I.J.M., Bokkers, E.A.M. Assessment time of the Welfare Quality® protocol for dairy cattle. Animal Welfare 2013, 22(1), 85-93.*
10. *Robichaud M.V., Rushen J., de Passillé A. M., Vasseur E., Orsel K., Pellerin D. Associations between on-farm animal welfare indicators and productivity and profitability on Canadian dairies: I. On freestall farms. Journal of Dairy Science 2019, 102(5), 4341-4351.*
11. *Robichaud M.V., Rushen J., de Passillé A.M., Vasseur E., Haley D., Pellerin D.nAssociations between on-farm cow welfare indicators and productivity and profitability on Canadian dairies: II. On tiestall farms. Journal of Dairy Science 2019, 102(5), 4352-4363.*
12. *Cincović M. Metabolički stress krava. Monografija. Poljoprivredni fakultet - Departman za veterinarsku medicinu, Novi Sad, 2016.*
13. *de Vries M., Bokkers E.A.M., Dijkstra T., Van Schaik, G., De Boer, I.J.M. Invited review: Associations between variables of routine herd data and dairy cattle welfare indicators. Journal of Dairy Science 2011, 94(7), 3213-3228.*
14. *Rushen J., Chapinal N., de Passile A.M. Automated monitoring of behavioural-based animal welfare indicators. Animal Welfare 2012, 21(3), 339-350.*
15. *Vasseur E., Gibbons J., Rushen J., De Passille, A. M. Development and implementation of a training program to ensure high repeatability of body condition scoring of dairy cows. Journal of Dairy Science 2013, 96(7), 4725-4737.*

16. *Gibbons J.M., Lawrence A.B., Haskell M.J. Measuring sociability in dairy cows. Applied Animal Behaviour Science 2010, 122(2-4), 84-91.*
17. *Martello L.S., Savastano Junior H., Silva S.L., Balieiro J.C.C. Alternative body sites for heat stress measurement in milking cows under tropical conditions and their relationship to the thermal discomfort of the animals. International Journal of Biometeorology 2010, 54, 647-652.*
18. *Herbut P., Angrecka S., Walczak J. Environmental parameters to assessing of heat stress in dairy cattle—a review. International journal of biometeorology 2018, 62, 2089-2097.*
19. *Gómez Y., Bieler R., Hankele A. K., Zähner M., Savary P., Hillmann E. Evaluation of visible eye white and maximum eye temperature as non-invasive indicators of stress in dairy cows. Applied Animal Behaviour Science 2018, 198, 1-8.*
20. *Kovacs L., Kezer F.L., Jurkovich V., Kulcsar-Huszenicza M., Tőzsér J. Heart rate variability as an indicator of chronic stress caused by lameness in dairy cows. PloS one 2015, 10(8), e0134792.*
21. *Sharma A., Umapathy G., Kumar V., Phillips C.J. Hair cortisol in sheltered cows and its association with other welfare indicators. Animals 2019, 9(5), 248.*
22. *Ebinghaus A., Knierim U., Simantke C., Palme R., Ivemeyer S. Fecal cortisol metabolites in dairy cows: a cross-sectional exploration of associations with animal, stockperson, and farm characteristics. Animals 2020, 10(10), 1787.*
23. *Jerram L.J., Van Winden S., Fowkes R.C. Minimally Invasive Markers of Stress and Production Parameters in Dairy Cows before and after the Installation of a Voluntary Milking System. Animals (Basel) 2020, Mar 31;10(4):589*
24. *Grelet C., Vanden Dries V., Leblois J., Wavreille J., Mirabito L., Soyeurt H., Franceschini S., Gengler N., Brostaux Y., Dehareng F. Identification of chronic stress biomarkers in dairy cows. Animal 2022, 16(5), 100502.*
25. *Alvåsen K., Mörk M.J., Sandgren C.H., Thomsen P.T., Emanuelson U. Herd-level risk factors associated with cow mortality in Swedish dairy herds. Journal of Dairy Science 2012, 95(8), 4352-4362.*
26. *Rilanto T., Reimus K., Orro, T., Emanuelson U., Viltrop A., Mõtus K. Culling reasons and risk factors in Estonian dairy cows. BMC Veterinary Research 2020, 16, 1-16.*
27. *Bahrami-Yekdangi M., Ghorbani G.R., Sadeghi-Sefidmazgi A., Mahnani A., Drackley J.K., Ghaffari M.H. Identification of cow-level risk factors and associations of selected blood macro-minerals at parturition with dystocia and stillbirth in Holstein dairy cows. Scientific reports 2022, 12(1), 5929.*
28. *Des Roches A.D.B., Faure M., Lussert A., Herry V., Rainard P., Durand D., Foucras G. Behavioral and patho-physiological response as possible signs of pain in dairy cows during Escherichia coli mastitis: A pilot study. Journal of Dairy Science 2017, 100(10), 8385-8397.*
29. *Konvičná J., Vargová M., Paulíková I., Kováč, G., Kostecká Z. Oxidative stress and antioxidant status in dairy cows during prepartal and postpartal periods. Acta Veterinaria Brno 2015, 84(2), 133-140.*
30. *Cincović R.M., Belić B., Radojičić B., Hristov S., Đoković R. Influence of lipolysis and ketogenesis to metabolic and hematological parameters in dairy cows during periparturient period. Acta veterinaria 2012, 62(4), 429-444.*
31. *Trevisi E., Amadori M., Cogrossi S., Razzuoli E., Bertoni G. Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. Research in Veterinary Science 2012, 93(2), 695-704.*
32. *Belić B., Cincović M., Lakić I., Đoković R., Petrović M., Ježek J., Starič, J. Metabolic status of dairy cows grouped by anabolic and catabolic indicators of metabolic stress in early lactation. Acta Scientiae Veterinariae 2018, 46, 1-9.*
33. *Ježek J., Cincović M. R., Nemec M., Belić B., Djoković R., Klinkon M., Starič, J. Beta-hydroxybutyrate in milk as screening test for subclinical ketosis in dairy cows. Polish journal of veterinary sciences 2017, 507-512.*
34. *Heirbaut S., Jing X.P., Stefańska B., Pruszyńska-Oszmałek E., Buysse L., Lutakome P., Fievez, V. Diagnostic milk biomarkers for predicting the metabolic health status of dairy cattle during early lactation. Journal of Dairy Science 2023, 106(1), 690-702.*
35. *Krnjaić S., Cincović M., Djoković R., Belić B., Ježek J., Starič J. The Influence of Energy Balance, Lipolysis and Ketogenesis on Metabolic Adaptation in Cows Milked Twice and Three Times Daily. Metabolites 2022, 12(11), 1090.*
36. *Wang H., Guo T., Wang Z., Xiao J., Gao L., Gao X., Wang H. PreCowKetosis: A Shiny web application for predicting the risk of ketosis in dairy cows using prenatal indicators. Computers and Electronics in Agriculture 2023, 206, 107697.*
37. *Džermeikaitė K., Bačėninaitė D., Antanaitis R. Innovations in Cattle Farming: Application of Innovative Technologies and Sensors in the Diagnosis of Diseases. Animals 2023, 13(5), 780.*
38. *Welfare Quality®. Welfare Quality® applied to growing and finishing pigs. In: Dalmau, A., Velarde, A., Scott, K., Edwards, S., Veissier, I., Keeling, L., Butterworth, A. (Eds.), Welfare Quality® Assessment Protocol for Pigs. Welfare Quality® Consortium, The Netherlands, 2009.*

39. Andreasen S, SandØe P, Forkman B. Can animal-based welfare assessment be simplified? A comparison of the Welfare Quality® protocol for dairy cattle and the simpler and less time-consuming protocol developed by the Danish Cattle Federation. Animal Welfare 2014, 23(1):81-94. doi:10.7120/09627286.23.1.081
40. EFSA. Scientific Opinion on the welfare of pigs on farm. EFSA Journal 2022; 20(8):7421, 319.
41. Vitali M., Santacroce E., Correa F., Salvarani C., Maramotti F. P., Padalino B., Trevisi P. On-farm welfare assessment protocol for suckling piglets: a pilot study. Animals 2020, 10(6), 1016.
42. Classyfarm Protocol - Alborali Loris G.; Bertocchi L. Benessere Animale: Linee Guida per la Categorizzazione del Rischio Nell'Allevamento Suino Dallo Svezzamento All'Ingrasso. Available online: http://www.classyfarm.it/ (accessed on 5 September 2023).
43. AssureWel. (2023): AssureWel Protocol for Pigs and Dry Sows: Lameness. Available online: http://www.assurewel. org/pigs/lamenes (accessed on 6 September 2023).
44. Renggaman A., Choi H.L., Sudiarto S.I., Alasaarela L., & Nam, O.S. (2015): Development of pig welfare assessment protocol integrating animal-, environment-, and management-based measures. Journal of animal science and technology, 57(1), 1-11.
45. Brandt, P., Rousing, T., Herskin, M.S., Olsen, E.V., Aaslyng, M.D. Development of an index for the assessment of welfare of finishing pigs from farm to slaughter based on expert opinion. Livestock Science 2017, 198, 65-71.
46. Villarroel M., Barreiro P., Kettlewell P., Farish M., Mitchell, M. Time derivatives in air temperature and enthalpy as non-invasive welfare indicators during long distance animal transport. Biosystems Engineering 2011, 110(3), 253-260.
47. Vermeer H.M., de Greef K.H., Houwers H.W.J. Space allowance and pen size affect welfare indicators and performance of growing pigs under Comfort Class conditions. Livestock Science 2014, (159): 79-86.
48. Fu L., Li H., Liang T., Zhou B., Chu Q., Schinckel A. P., Huang R. Stocking density affects welfare indicators of growing pigs of different group sizes after regrouping. Applied Animal Behaviour Science 2016, 174, 42-50.
49. Meyer-Hamme S., Lambertz C., Gauly M. Does group size have an impact on welfare indicators in fattening pigs? Animal 2016, 10(1), 142-149.
50. Andersen I., Ocepek M., Thingnes S., Newberry R. Welfare and performance of finishing pigs on commercial farms: Associations with group size, floor space per pig and feed type. Applied Animal Behaviour Science 2023, 265. 105979.
51. Plut J., Snoj T., Golinar Oven I., Štukelj M. The Combination of Serum and Oral Fluid Cortisol Levels and Welfare Quality Protocol® for Assessment of Pig Welfare on Intensive Farms. Agriculture 2023, 13(2), 351.
52. Vermeer H. M., Hopster H. Operationalizing principle-based standards for animal welfare—indicators for climate problems in pig houses. Animals 2018, 8(4), 44.
53. Pierozan C. R., Dias C. P., Temple D., Manteca X., da Silva C.A. Welfare indicators associated with feed conversion ratio and daily feed intake of growing-finishing pigs. Animal Production Science 2020, 61(4), 412-422.
54. Wegner B., Spiekermeier I., Nienhoff H., Große-Kleimann J., Rohn, K., Meyer, H., Plate H., Gerhardy H., Kreienbrock L., Beilage E.G., Kemper, N. Application of the voluntary human approach test on commercial pig fattening farms: a meaningful tool?. Porcine health management 2020, 6(1), 1-13.
55. Wiseman-Orr M. L., Scott E. M., & Nolan, A. M. (2011): Development and testing of a novel instrument to measure health-related quality of life (HRQL) of farmed pigs and promote welfare enhancement (Part 1). Animal Welfare, 20(4), 535-548.
56. Telkanranta H., Marchant-Forde J.N., Valros A. Tear staining in pigs: a potential tool for welfare assessment on commercial farms. Animal 2016, 10(2), 318-325.
57. Valros A., Välimäki E., Nordgren H., Vugts J., Fàbrega E., Heinonen M. Intact tails as a welfare indicator in finishing pigs? Scoring of tail lesions and defining intact tails in undocked pigs at the abattoir. Frontiers in Veterinary Science 2020, 7, 405.
58. Diana A., Boyle L.A., García Manzanilla E., Leonard F.C., Calderón Díaz J.A. Ear, tail and skin lesions vary according to different production flows in a farrow-to-finish pig farm. Porcine Health Management 2019, 5, 1-9.
59. Lawrence A.B., Vigors B., Sandøe P. What is so positive about positive animal welfare?—a critical review of the literature. Animals 2019, 9(10), 783.
60. Rowe E., Mullan S. Advancing a "Good Life" for farm animals: development of resource tier frameworks for on-farm assessment of positive welfare for beef cattle, broiler chicken and pigs. Animals 2022, 12(5), 565.
61. Mellor D.J. Enhancing animal welfare by creating opportunities for positive affective engagement. New Zealand Veterinary Journal 2015, 63(1), 3-8.
62. Papageorgiou M. Positive welfare indicators of the domestic pig (Sus scrofa domesticus), a review: Which of the indicators are being used for the on-farm evaluation of pig's positive welfare? Master Thesis, Linnæus University, Sweden, p. 38, 2022.
63. Mellor D.J. Positive animal welfare states and encouraging environment-focused and animal-to-animal interactive behaviours. New Zealand Veterinary Journal 2015, 63(1), 9-16.

ISBN 978-86-7520-611-8

64. Brown S.M., Klaffenböck M., Nevison I.M., Lawrence A.B. Evidence for litter differences in play behaviour in pre-weaned pigs. Applied Animal Behaviour Science 2015, 172, 17-25.
65. Brown S.M., Peters R., Nevison I.M., Lawrence A.B. Playful pigs: Evidence of consistency and change in play depending on litter and developmental stage. Applied animal behaviour science 2017, 198, 36-43.
66. Martin J. E., Ison S. H., Baxter E.M. The influence of neonatal environment on piglet play behaviour and post-weaning social and cognitive development. Applied Animal Behaviour Science 2015, 163, 69-79.
67. Yang C. H., Ko H. L., Salazar L. C., Llonch L., Manteca X., Camerlink I., Llonch P. Pre-weaning environmental enrichment increases piglets' object play behaviour on a large scale commercial pig farm. Applied Animal Behaviour Science 2018, 202, 7-12.
68. Marcet Rius M., Cozzi A., Bienboire-Frosini C., Teruel E., Chabaud C., Monneret P., Leclercq J., Lafont-Lecuelle C., Pageat P. Selection of putative indicators of positive emotions triggered by object and social play in mini-pigs. Applied Animal Behaviour Science 2018, 202, 13-19.
69. Ahloy-Dallaire J., Espinosa J., Mason G. Play and optimal welfare: does play behavior indicate the presence of positive affective states? Behavioral Processes 2018, 156, 3-18.
70. Keeling L. Indicators of good welfare. In: Chun Choe, J. (ed) Encyclopedia of Animal Behavior. Volume 1. Second Edition. London. UK: Elsevier, 2019.
71. Lawrence A.B., Newberry R.C., Špinka M. Positive welfare: what does it add to the debate over pig welfare? Chapter 15. pp. 415-444 in Advances in Pig Welfare. 1st edition. Ed by M Špinka, Woodhead Publishing (Elsevier), Duxford, UK, 2018.
72. Spinka M. Behaviour of Pigs. In: Jensen, P. (ed) The Ethology of Domestic Animals: An Introductory Text. Wallingford, Oxfordshire, UK; Boston, MA: CABI. pp. 214-227, 2017.
73. Rault J.L. Be kind to others: Prosocial behaviours and their implications for animal welfare. Applied Animal Behaviour Science 2019, 210, 113-123.
74. Marcet Rius M., Pageat P., Bienboire-Frosini C., Teruel E., Monneret P., Leclercq J., Lafont-Lecuelle C., Cozzi A. Tail and ear movements as possible indicators of emotions in pigs. Applied Animal Behaviour Science 2018, 205, 14-18.
75. Marcet-Rius M., Fàbrega E., Cozzi A., Bienboire-Frosini C., Descout E., Velarde A., Pageat P. Are tail and ear movements indicators of emotions in tail-docked pigs in response to environmental enrichment? Animals 2019, 9(7), 449.
76. Laurijs K.A., Briefer F.E., Inonge R., Webb L.E. Vocalisatios in farm animals: A step towards positive welfare assessment. Applied animal behavior science 2021, 236, 105264.
77. Vullo C., Barbieri S., Catone G., Graïc J., Magaletti M., Di Rosa A., Motta A., Tremolada C., Canali E., Dalla Costa E. Is the Piglet Grimace Scale (PGS) a Useful Welfare Indicator to Assess Pain after Cryptorchidectomy in Growing Pigs? Animals 2020, 10(3), 412.
78. Hansen M.F., Baxter E.M., Rutherford K.M., Futro A., Smith M.L., Smith L.N. Towards facial expression recognition for on-farm welfare assessment in pigs. Agriculture 2021, 11(9), 847.
79. Vigors B., Lawrence A. What are the positives? exploring positive welfare indicators in a qualitative interview study with livestock farmers. Animals 2019, 9(9), 694.
80. van Staaveren N., Doyle B., Manzanilla E. G., Calderón Díaz J. A., Hanlon A., Boyle L.A. Validation of carcass lesions as indicators for on-farm health and welfare of pigs. Journal of Animal Science 2017, 95(4), 1528-1536.
81. Matthews S.G., Miller A. L., Plötz T., Kyriazakis I. Automated tracking to measure behavioural changes in pigs for health and welfare monitoring. Scientific reports 2017, 7(1), 17582.
82. Amos N., Sullivan R., van de Weerd H. The Business Benchmark on Farm Animal Welfare Methodology Report. (2018) (Available online: https://www.bbfaw.com/media/1555/bbfaw_methodology_report_2018.pdf (accessed on 5 September ,2023), 2018.
83. Sandoe P., Hansen H.O., Rhode H.L.H., Houe H., Palmer C., Forkman B., Christensen T. Benchmarking farm animal welfare—A novel tool for cross-country comparison applied to pig production and pork consumption. Animals 2020, 10(6), 955.
84. World Animal Protection. Animal Protection Index. Available online: https://api.worldanimalprotection.org/# (accessed on 24 April 2020), 2020.
85. Dalmau A., Temple D., Rodríguez P., Llonch P., Velarde A. Application of the Welfare Quality® protocol at pig slaughterhouses. Animal Welfare 2009, 18:497–505.
86. Dalmau A., Fabrega E., Manteca X., Velarde A. Health and Welfare Management of Pigs Based on Slaughter Line Records. J Dairy Vet Anim Res 2014, 1(3): 00016.
87. Nielsen A.C. Data warehouse for assessing animal health, welfare, risk management and-communication. Acta Veterinaria Scandinavica, 53(Suppl 1):S3, 2011.
88. Knage-Rasmussen K.M., Rousing T., Sorensen J.T., Houe H. Assessing animal welfare in sow herds using data on meat inspection, medication and mortality. Animal 2015, 9:509–515.

89. *Nyman A.K., Lindberg A., Sandgren C.H. Can precollected register data be used to identify dairy herds with good cattle welfare? Acta Veterinaria Scandinavica 2011, 53(Suppl 1):S8.*
90. *de Vries M., Bokkers E.A.M., van Schaik G., Engel B., Dijkstra T., de Boer I.J.M.. Exploring the value of routinely collected herd data for estimating dairy cattle welfare. Journal of Dairy Science 2014, 97:715–730.*
91. *Krug C., Haskell M.J., Nunes T., Stilwell G. Creating a model to detect dairy cattle farms with poor welfare using a national database. Preventive Veterinary Medicine 2015, 122:280–286.*
92. *EFSA. Scientific Opinion on the public health hazards to be covered by inspection of meat (swine). EFSA Journal 2011, 9(10):2351.*
93. *Harley S., More L., Boyle S., O'Connell N., Hanlon A. Good animal welfare makes economic sense: Potential of pig abattoir meat inspection as a welfare surveillance tool. Irish Veterinary Journal 2012, 65:11–22.*
94. *Stärk K.D.C., Alonso S., Dadios N., Dupuy C., Ellerbroek L., Georgiev M., Hardstaff J., Huneau-Salaün A., Laugier C., Mateus A., Nigsch A., Afonso A., Lindberg A. Strengths and weaknesses of meat inspection as a contribution to animal health and welfare surveillance. Food Control 2014, 39:154–162.*
95. *Friedrich L., Krieter J., Kemper N., Czycholl I. Iceberg indicators for sow and piglet welfare. Sustainability 2020, 12(21), 8967.*
96. *EFSA. Scientific Opinion on the use of animal-based measures to assess welfare in pigs. EFSA Journal 2012, 10 (1):2512.*
97. *van Staaveren N., Doyle B., Hanlon A., Boyle L.A. Multi-stakeholder focus groups on potential for meat inspection data to inform management of pig health and welfare on farm. Agriculture 2019, 9(2), 40.*
98. *Candiani D., Salamano G., Mellia E., Doglione L., Bruno R., Toussaint M., Gruys E. A combination of behavioral and physiological indicators for assessing pig welfare on the farm. Journal of Applied Animal Welfare Science 2008, 11(1), 1-13.*
99. *Valros A., Munsterhjelm C., Puolanne E., Ruusunen M., Heinonen M., Peltoniemi O. A., Pösö A.R. Physiological indicators of stress and meat and carcass characteristics in tail bitten slaughter pigs. Acta Veterinaria Scandinavica 2013, 55, 1-8.*
100. *Valros A., Ahlström S., Rintala H., Häkkinen T., Saloniemi H. The prevalence of tail damage in slaughter pigs in Finland and associations to carcass condemnations. Acta Agriculturae Scandinavica 2004, Section A, Animal Science, 54:213–219.*
101. *EFSA. Scientific opinion of the panel on animal health and welfare on a request from commission on the risks associated with tail biting in pigs and possible means to reduce the need for tail docking considering the different housing and husbandry systems. EFSA Journal 2007, 611:1–13.*
102. *Keeling L., Larsen A. What are the characteristics of tail biting pigs. Proc. Svenska djurhalsovardens fortbildningkonferens, Billingen, Skovde, Sweden, 2004.*
103. *Wallgren P., Lindahl E. The influence of tail biting on performance of fattening pigs Acta Veterinaria Scandinavica 1996, 37:453–460.*
104. *Walker P.K., Bilkei G.J. Tail-biting in outdoor pig production. The Veterinary Journal 2006, 171(2), 367-369.*
105. *Fraser D., Phillips P.A., Thompson B.K., Tennesen T. Effect of straw on the behaviour of growing pigs. Applied Animal Behavior Science 1991, 30:307–318.*
106. *Beattie V.E., Walker N., Sneddon I.A. Effects of environmental enrichment on behaviour and productivity of growing pigs. Animal Welfare 1995, 4:207–220.*
107. *Klont R.E., Hulsegge B., Hoving-Bolink A.H., Gerritzen M.A., Kurt E., Winkelman-Goedhart H.A., De Jong I.C., Kranen R.W. Relationships between behavioral and meat quality characteristics of pigs raised under barren and enriched housing conditions. J Anim Sci 2010, 79:2835–2843.*
108. *Broom D.M. A review of animal welfare measurement in pigs. Pig News Info 1996, 17:109–114.*
109. *Schrøder-Petersen D., & Simonsen H.B. Tail biting in pigs. Veterinary Journal 2001, 162:196–210.*
110. *Munsterhjelm C., Brunberg E., Heinonen M., Keeling L., Valros A. Stress measures in tail biters and bitten pigs in a matched case-control study. Animal Welfare 2013, 22(3), 331-338.*
111. *Schrader L., Ladewig J. Temporal differences in the response of the pituitary adrenocortical axis, the sympatho-adrenomedullar axis, heart rate, and behaviour to a repeated daily stressor in domestic pigs. Physiology & Behavior 1999, 66:775–783.*
112. *Ruis M.A.W., Te Brake J.H.A., Engel B, Buist W.G., Blokhuis H.J., Koolhaus JM. Adaptation to social isolation acute and long-term stress responses of growing gilts with different coping characteristics. Physiology & Behavior 2001, 73:541–551.*
113. *Fries E., Hesse J., Hellhammer J., Hellhammer D.H. A new view on hypocortisolism. Psychoneuroendocrinology 2005, 30:1010–1016.*
114. *de Groot J., De Jong I.C., Prelle I.T., Koolhaus J.M. Immunity in barren and enriched housed pigs differing in baseline cortisol concentration. Physiology & Behavior 2000, 71:217–223.*

115. *Kanitz E., Otten W., Tuchscherer M. Central and pheripheral effects of repeated noise stress on hypothalamic-pituitary-adrenocortical axis in pigs. Livestock Production Science 2005, 94:213–224.*
116. *Munsterhjelm C., Valros A., Heinonen M., Hälli O., Siljander-Rasi H., Peltoniemi O.A.T. Environmental enrichment in early life affects cortisol patterns in growing pigs. Animal 2010, 4:242–249.*
117. *Warriss P.D., Brown S.N., Edwards J.E., Knowles T.G. Effect of lairage time on level of stress and meat quality. Animal Science 1998, 66:255–261.*
118. *Henckel P., Karlsson A., Jensen M.T., Oksbjerg N., Søholm Petersen J. Metabolic conditions in Porcine longissimus muscle immediately pre-slaughter and its influence on peri-and post mortem energy metabolism. Meat Science 2002, 62:145–155.*
119. *Guàrdia M.D., Estany J., Balash S., Oliver M.A., Gispert M., Diestre A. Risk assessment of DFD meat due to the pre-slaughter conditions in pigs. Meat Science 2005, 70:709–716.*
120. *Liu Y., Steinacker J.M. Changes in skeletal muscle heat shock proteins: pathological significance. Frontiers in Bioscience 2001, 6:12–25.*
121. *Sepponen K., Pösö AR. The inducible form of heat shock protein 70 in the serum, colon and small intestine of the pig: comparison to conventional stress markers. Veterinary Journal 2006, 171:519–524.*
122. *Faucitano L., Chevillon P., Ellis M. Effects of feed withdrawal prior to slaughter and nutrition on stomach weight, and carcass and meat quality in pigs. Livestock Science 2010, 127(2-3), 110-114.*
123. *Eikelenboom G., Bolink A.H., Sybesma W. Effects of feed withdrawal before delivery on pork quality and carcass yield. Meat Science 1991, 29(1), 25-30.*
124. *Fraqueza M. J., Roseiro L. C., Almeida J., Matias E., Santos C., Randall, J.M. Effects of lairage temperature and holding time on pig behaviour and on carcass and meat quality. Applied Animal Behaviour Science 1998, 60(4), 317-330.*
125. *Varón-Álvarez L. J., Romero M. H., Sánchez J. A. Caracterización de las contusiones cutáneas e identificación de factores de riesgo durante el manejo presacrificio de cerdos comerciales. Archivos de medicina veterinaria 2014, 46(1), 93-101.*
126. *Guàrdia M. D., Estany J., Balasch S., Oliver M. A., Gispert M., Diestre, A. Risk assessment of skin damage due to pre-slaughter conditions and RYR1 gene in pigs. Meat Science 2009, 81(4), 745-751.*
127. *Martín-Peláez S., Peralta B., Creus E., Dalmau A., Velarde A., Pérez J.F., Mateu E., Martín–Orúe S.M. Different feed withdrawal times before slaughter influence caecal fermentation and faecal Salmonella shedding in pigs. The Veterinary Journal 2009, 182(3), 469-473.*
128. *Dokmanović M., Velarde A., Tomović V., Glamočlija N., Marković R., Janjić J., Baltić M. Ž. The effects of lairage time and handling procedure prior to slaughter on stress and meat quality parameters in pigs. Meat Science 2014, 98(2), 220-226.*
129. *Blomke L., Volkmann N., Kemper N. Evaluation of an automated assessment system for ear and tail lesions as animal welfare indicators in pigs at slaughter. Meat Science 2020, 159, 107934.*
130. *Camerlink I., Ursinus W. Tail postures and tail motion in pigs: A review. Applied Animal Behavior Science 2020, 230, 105079.*
131. *Courboulay V., Meunier-Salaün M.C., Stankowiak M., Pol, F. BEEP: An advisory pig welfare assessment tool developed by farmers for farmers. Livestock Science 2020, 240, 104107.*
132. *Gomez Y., Stygar A. H., Boumans I. J., Bokkers E. A., Pedersen L. J., Niemi J. K., Llonch, P. A systematic review on validated precision livestock farming technologies for pig production and its potential to assess animal welfare. Frontiers in Veterinary Science 2021, 8, 660565.*
133. *Haigh A., Chou J.Y., O'Driscoll, K. Variations in the behavior of Pigs during an open field and novel object test. Frontiers in Veterinary Science 2020, 7, 607.*
134. *Larsen M.L., Wang M., Norton T. Information technologies for welfare monitoring in pigs and their relation to Welfare Quality®. Sustainability 2021, 13(2), 692.*
135. *Michelsen A.M., Hakansson F., Pedersen Lund V., Kirchner M.K., Otten N.D., Denwood M., Rousing T., Houe H., Forkman B. Identifying areas of animal welfare concern in different production stages in Danish pig herds using the Danish Animal Welfare Index (DAWIN). Animal Welfare 2023, 32, e47, 1–11.*
136. *Pfeifer M., Koch A., Lensches C., Schmitt A. O., Hessel E.F. Acceptance and feasibility of a guideline for the animal welfare assessment of fattening pigs from farmers' point of view. Animals 2020, 10(4), 711.*
137. *Statham P., Hannuna S., Jones S., Campbell N., Robert Colborne G., Browne W.J., Paul E.S., Mendl M. Quantifying defence cascade responses as indicators of pig affect and welfare using computer vision methods. Scientific reports 2020, 10(1), 8933.*

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Article
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BIOSECURITY MEASURES ON RUMINANT FARMS

BIOSIGURNOSNE MERE NA FARMAMA PREŽIVARA

Slavča Hristov[a*], Branislav Stanković[a], Jože Starič[b], Dimitar Nakov[c], Jasna Prodanov-Radulović[d], Bojan Milovanović[e], Ilias Chantziaras [f], Alberto Allepuz [g]

[a]University of Belgrade - Faculty of Agriculture, Belgrade-Zemun, Republic of Serbia
[b]University of Ljubljana, Veterinary Faculty, Ljubljana, Slovenia
[c]Faculty of Agriculture, Goce Delcev University – Stip, Stip, Republic of North Macedonia
[d]Scientific Veterinary Institute “Novi Sad”, Novi Sad, Republic of Serbia,
[e]Institute of Veterinary Medicine of Serbia, Belgrade, Republic of Serbia
[f]Veterinary Epidemiology Unit, Faculty of Veterinary Medicine, Ghent University, Belgium
[g]Department of Animal Health and Anatomy, Universitat Autònoma de Barcelona, Spain
*Corresponding author: hristovs@agrif.bg.ac.rs

S.H. https://orcid.org/0000-0002-1719-9971, B.S. https://orcid.org/0000-0003-3925-6102, J.S. https://orcid.org/0000-0002-5156-5277, D.N. https://orcid.org/0000-0001-6159-5595, J.P.R. https://orcid.org/0000-0002-6116-3271, B.M. https://orcid.org/0000-0001-8573-0771, I.C. https://orcid.org/0000-0001-5687-9577, A.A. https://orcid.org/0000-0003-3518-1991

ABSTRACT

In many scientific papers, the term biosecurity measures (BSMs) are defined as the implementation of segregation, sanitation or management procedures specifically designed to reduce the likelihood of the introduction, establishment, survival or spread of a potential pathogen into, within or from a farm or geographical area. The main BSMs (general external and internal BSMs related to newly introduced animals, farm workers, family members, visitors and service providers, vehicles, tools and equipment, location of farms, water and feed, control programs, management practices, handling of raw materials, work procedures, training, plans and records), based on literature data, guides, instructions, recommendation codes and checklists, are presented in the paper. In addition to the BSMs mentioned, the importance of segregation, cleaning and disinfection is emphasized. The most important and effective part of biosecurity is to keep infected animals and contaminated material away from non-infected animals. Cleaning and disinfecting barns, vehicles and equipment, especially boots and clothing, is a very effective way to minimize the transmission of disease to or between animals. It is very important to implement BSMs as a long-standing and successful practice on farms to maintain animal health. These measures should be included in a comprehensive biosecurity plan, which is tailored to farms characteristics and needs that must be fully implemented. A biosecurity plan and the design and implementation of biosecurity programs should address how farmers handle animals, vehicles and human access to the farm, as well as animal health and work procedures. Key BSMs should be followed on an ongoing basis and, working with veterinarians, farmers themselves can play an important role in keeping animals and production as healthy as possible. It is important to regularly assess the implementation of BSMs using appropriate questionnaires, which can highlight deficiencies that should be addressed immediately.

Keywords: ruminants, BSMs, biosecurity plan and program, questionnaire

INTRODUCTION

Nowadays, biosecurity is clearly defined and considered as a central part of the One Health concept. However, although the term biosecurity measures (BSMs) has been used for years, there is still no consistent definition. According to the Food and Agriculture Organization of the United Nations, "biosecurity is a strategic and integrated concept that encompasses the policy and regulatory frameworks

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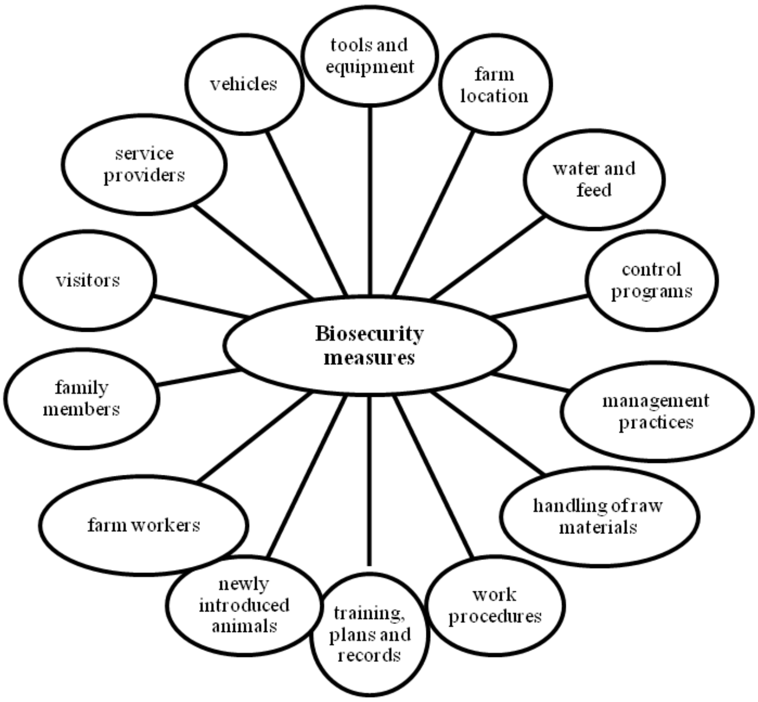
(including tools and activities) used to analyse and manage risks in the areas of food safety, public health, animal life and health, and plant life and health, including associated environmental risks." (1).

Considering that the unclear definition of BSMs, especially at the farm and policy level, can lead to misunderstandings, low acceptance, poor implementation and thus suboptimal biosecurity along the food production chain, Huber et al. (2) defined BSM as the implementation of a segregation, sanitation or management procedure specifically aimed at reducing the likelihood of the introduction, establishment, survival or spread of potential pathogens into, within or from a farm or geographical area.

This paper aims to familiarize field veterinarians and farmers with the most important BSMs, i.e. essentially the most important general external and internal BSMs related to, newly introduced animals, farm workers, family members, visitors and service providers, vehicles, tools and equipment, the farm location, water and feed, control programs, management practices, handling of raw materials, work procedures, training, plans and records, which would help them to increase the knowledge, motivate stakeholedrs and improve cooperation.

BIOSECURITY MEASURES

Graph 1 shows biosecurity measures related to newly introduced animals, farm workers, family members, visitors and service providers, vehicles, tools and equipment, farm location, water and feed, control programs, management practices, raw material handling, work procedures, training, plans and records.



Graph 1. Biosecurity measures on ruminant farms

INTRODUCTION, ISOLATION AND MOVEMENT OF ANIMALS

Movements of domestic and wild animal populations are important for the spread of disease. Many recent examples of disease spread are due to the intentional movement of livestock or wildlife. Understanding the extent of these movements and the associated risks is fundamental to clarifying the epidemiology of these diseases, some of which may pose zoonotic risks. The importance of global animal trade is reviewed and the role of unregulated trade in animals is highlighted. Several key examples are discussed where animal movements have introduced pathogens into previously disease-free areas. Measures based on enhanced surveillance

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are proposed to mitigate the risks of introducing new pathogens (3). It is important to consider animal movement contacts between holdings of different production types (4, 5), the knowledge, attitude, and practice of nomadic and semi-nomadic pastoralists (6, 7) and the role of auction markets in cattle movements (8).

Numerous studies have shown that the introduction of new animals to the farm and the movement of animals (transport and reintroduction of animals) plays an important role in the spread of disease (3, 8 - 11), which is why maintaining a closed herd is one of the most important BSMs in disease prevention (3-5, 10, 12 - 15). Trade in small ruminants and their products is of great importance for biosecurity, as sheep and goats can transmit zoonotic diseases such as Rift Valley fever, Crimean-Congo hemorrhagic fever, brucellosis and listeriosis (16). Therefore, management procedures related to the introduction and movement of animals in a way that minimizes the risk of introducing or spreading infectious diseases are of paramount importance.

The review by Mee et al. (15) discusses evidence for many transmission routes as an introduction of infectious agents onto dairy and beef farms, which can occur through direct transmission (purchased cattle, reintroduced native cattle and contact with neighbouring cattle) or indirect transmission (faeces, visitors, other species and biological material). In the absence of eradication programs for many endemic infectious diseases, bioexclusion is the most important management technique for risk reduction. The greatest risk for the introduction of infectious agents is posed by the purchase of animals where the new arrivals are in direct contact with the recipient herd over a longer time (11).

As newly purchased animals entering the farm pose a high risk for the unintentional introduction of pathogens, it is necessary to check the health status of the animals before purchase. This means firstly that the animals should only be purchased from farms with the same or higher health status, then the animals should not be purchased from herds with lameness, abscesses, sore mouth, ringworm, cloudy eyes or other clinical signs of disease, and the animals intended for purchase should be examined, paying particular attention to misshapen, deformed or recently pared hooves. Care should also be taken to ensure that animals coming from areas where there are known problems with anthelmintic-resistant worms are dewormed and faecal tested before arrival to ensure that no resistant parasites are introduced into the herd (16 - 23).

Segregation, observation and, if necessary, treatment of newly introduced animals in quarantine, i.e. isolation in separate pens depending on the situation, separation of the quarantine facilities from the area where the rest of the herd is kept, testing of animals for certain diseases in the quarantine facilities, quarantine of all newly arrived animals for a certain period and preferably until after birth, should be implemented. In addition, newly arrived animals must be routinely dewormed and all disease tests, treatments, procedures and vaccinations must be completed before the animals are released from isolation. Quarantine paddocks or pens should be located as close as possible to the farm entrance and away from other animals. There should be a double fence with a minimum distance of 3 metres between the newly arrived animals and the resident animals. In general, farmers should bear in mind that it is best to rear as many replacement animals as possible on their farm and only add new animals from other farms if necessary (10, 18 - 24).

When transporting animals to exhibitions, fairs and sales, it must be borne in mind that the transported animals can also transmit diseases to other animals through mixing or contact with pens, vehicles, people and equipment. To prevent the transmission of diseases in this way, animals must not leave the holding until they have been approved by the competent authority. It must also be ensured that all animals participating in a show or mela undergo a compulsory veterinary examination before unloading and that animals showing signs of disease are not unloaded. In addition, it should be ensured that animals that have recently given birth or have aborted or may give birth at the time of the show are excluded due to the risk of transmission of infectious abortive diseases. Animals should only be transported in a vehicle that has been cleaned and disinfected before use. It is important to avoid direct contact with other animals and to limit proximity to other animals during transportation and on-site. Farmer must bring bedding and feed from the farm and ensure a clean water supply on-site and bring feeders, water buckets and grooming and handling equipment from the farm. The handling of farm animals by other persons must be restricted. The welfare of the animals should be taken into account following transportation regulations when

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transporting animals that are sold and taken away from the farm (18 - 24).

Knowledge of the health status of animals returning to the farm (e.g. animals participating in shows, etc.) is of particular importance to minimize the risk of introducing and spreading diseases in the existing herd. With this in mind, it must be ensured that animals leaving and returning to the farm are separated for a certain period to assess their susceptibility to infection. This should take into account hygiene measures and contact with other animals during the absence. Returning animals should then be tested in consultation with a veterinarian before being housed or relocated (tests to determine disease status may include serology, culture and faecal egg count). The animal should remain in isolation until test results are available. Care should be taken during isolation to ensure that no clinical signs of the disease are detected and a plan should be put in place for animals with positive test results, e.g. treatment, no purchase or disposal, no sharing of feeding or watering facilities, pens, handling facilities or equipment of isolated and housed animals unless these have been previously cleaned and disinfected. Finally, all disease tests, treatments, procedures and vaccinations should be completed before animals are released from isolation (18 - 24).

In isolation and movement control, should be also considered that the farm is surrounded by a natural barrier or a fence, that the number of access points to the farm is minimized and access to the farm is restricted, that the movement of people and vehicles on and around the property is minimized, that contact with animals from other herds is not allowed during grazing, that measures are taken to prevent direct contact between animals and wildlife, and finally that measures are taken to prevent direct contact between ruminants and other animal species (20 - 23).

In addition, should be remembered to designate "approved access areas" for farm contractors (e.g. veterinarians, animal caretakers, artificial insemination staff, and hay suppliers), delivery and collection vehicles (e.g. milk tankers, livestock trucks and feed) and service personnel (e.g. supply technicians, officials). Also, delivery and collection vehicles (e.g. milk tankers, livestock trucks and feed) and service personnel (e.g. supply technicians, government officials) should be identified and signposted as necessary, and facilities should be provided in "approved access areas" for farm contractors and visitors to clean boots and equipment on arrival and before departure (20 - 23).

In studies of biosecurity on cattle farms in North West England, there were large differences between farms in the type and extent of BSMs. The majority of farmers did not isolate purchased animals, while a small proportion always isolated animals. Many farmers carried out post-movement treatments, mainly vaccinations and anthelmintics, but very few farms reported that they carried out post-movement health checks. In addition, there appeared to be wide variation in the level of BSMs carried out by the different companies and contractors that visited the farms. Rendering companies and contracted distributors of animal waste rarely disinfect themselves and their vehicles, even though the likelihood of contact with infectious agents is high. These findings suggest that while certain biosecurity practices are being implemented, many of them are rarely or never used. This may be due to many factors, including cost (time and money), lack of proven effectiveness of the practices, and lack of training of veterinarians, producers and other herd health professionals. If preventive medicine is to be fully utilized by the farming industry, further research into the reasons for the lack of uptake is essential (25).

FARM WORKERS, FAMILY MEMBERS, VISITORS AND SERVICE PROVIDERS

The people entering the farm are mainly farm workers, family members, visitors and service providers. It must be ensured that their activities and movements do not endanger the health of humans and animals. To ensure this, workers employed on the farm must take BSMs. This includes ensuring that all farm workers know and understand the biosecurity practices on the farm and are willing to implement them and also follow changes to the plan and practices. In addition, it must be ensured that in the event of a visit from service providers or visitors, all employees on the farm are informed. Numerous studies have shown that restricted access for visitors is a biosecurity measure that is not often applied (25 - 28).

The movement of owners, employees, visitors, veterinarians and service providers is commonplace on a farm and increases the risk of disease introduction and spread. Measures can be developed and implemented to reduce these risks. Access to farms should be restricted by establishing

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different zones with different levels of protection. These zones should be demarcated by boundaries and appropriate signage. The movement of people into a designated zone, out of a designated zone and between designated zones can be controlled through the use of controlled access points (22, 23)

Several important biosecurity principles should be followed as much as possible, i.e.: all personnel are exclusive to the farm (i.e., not shared with other farms), visitors who do not necessarily need to come into contact with the animals, such as salespeople, consultants, etc., are not allowed to have contact with the animals, visitors who need to come into contact with the animals, such as veterinarians, shearing personnel, hoof trimmers, etc., are provided with boots/shoe covers and overalls on the farm, place footbaths at the entrance to the premises, define and place signs where appropriate, ensure that working practices are designed to minimize the movement of people, vehicles or equipment into areas where animals are kept and keep records of visitors to the farm (19).

All visitors and service providers must be aware of and prepared for the applicable biosecurity practices before their visit. Upon entering the farm, all visitors and service providers must register their visit in the visitor register. Once registered, all those entering, working or visiting the farm must wash and disinfect their hands when entering and leaving the farm premises, when moving between areas, and when entering or exiting certain designated risk areas of the premises, such as isolation or sick bays. Hands should also be washed or disinfected before and after any contact with animals, especially sick animals or animals of unknown health status, after contact with potentially contaminated materials such as dead animals, aborted foetuses, placenta or manure (18, 21 - 24).

After washing hands at the entrance, visitors and service providers must be briefed about the layout of the farm, which areas they are permitted to access, and what biosecurity practices need to be applied in that location. In doing so, it is necessary to ensure that visitors and service providers access only areas of the farm that are necessary and to allow contact with animals only when necessary. It is of great importance to encourage the use of personal protective equipment when visitors and service providers move onto farm property. Also, visitors and service providers are to be explained about the use of personal protective equipment, how to put it on and remove it as well as where they should dispose of it (20 - 23).

In addition, should be remembered to meet with farm workers and their family members at least twice a year to discuss the benefits and effectiveness of each biosecurity plan practice. Basic things such as leaving the animal area without cleaning clothes of contaminants such as animal feces, or leaving the animal area without cleaning and sanitizing shoes may be discussed in such meetings (19, 22, 23).

VEHICLES, TOOLS AND EQUIPMENT

It would be difficult to control and monitor the spread of disease without restricting parking and vehicle movements within the premises. The biosecurity measure stipulates that vehicles moving from farm to farm may only enter the farm if they have been cleaned and disinfected in such a way that any contamination of the underbody or exterior of the vehicle cannot enter the farm. It should be borne in mind that several unsecured entrances to the farm make it difficult to monitor and control the entry of vehicles into the premises. Therefore, only one or two routes should be used to enter the premises.

Livestock transport vehicles should be loaded and unloaded at the perimeter of the premises and the animals can then be led to the isolation area. Feed vehicles should also be unloaded or loaded in designated areas, preferably close to the feed outlets, without entering the main area of the farm. It should be ensured that trucks used for the transportation of feed or silage are not used for purposes that pose a biosecurity risk to the herd and that they are cleaned appropriately before use (20 - 24).

Essentially, when transporting ruminants, only animals that are in a fit condition for loading are selected to minimize the potential spread of disease and/or contamination through transport, transport vehicles for animals, feed, supplies, maintenance, etc. are only allowed to enter and leave the farm premises if they are properly disinfected, and transport vehicles for removal (i.e. to slaughter, feed stores, etc.) arrive at the farm empty (i.e. without other animals), cleaned and disinfected. Particular care shall be taken to ensure that dead animals and/or animal materials are collected by a rendering vehicle and that the rendering vehicle can remove dead animals without entering the premises of the holding (20 - 23).

Cleaning and disinfection is the most important biosecurity tool for reducing the risk of

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vehicle-borne disease. A washing area should be provided for vehicles that must enter the farm premises. If possible, a high-pressure washing system should be used to clean vehicles and equipment, away from crops or animals. It must also be ensured that the wastewater from the washing facility is diverted away from the production areas of the farm. If it is not possible to secure the washing area, it must be ensured that vehicles entering the farm pass through a wheel washing facility installed at the entrance to the farm. Finally, it is important to keep a vehicle register in which all necessary information is properly entered when entering and leaving the facility (20 - 24).

The risk of disease spread is higher when equipment is borrowed, lent or purchased second-hand from other properties. It must be ensured that the movement and use of equipment and machinery does not endanger human and animal health in any way, not only within the farm but also in other farms in the vicinity. It is best to minimize the lending and borrowing of equipment between properties. If equipment is borrowed, it should be ensured that it is cleaned before and after use. We also need special tools, clothing and footwear available for specific areas such as production areas or isolation areas where sick or quarantined animals are kept. Cleaning and disinfection of needles, tools, etc., between each use, and especially between herds is of paramount importance (20 - 22).

LOCATION OF FARMS

The risk of disease increases many times over if the farm is located near other farms, slaughterhouses, livestock markets, waste disposal facilities and rendering centres (20 - 22). The location near animal transport routes and waterways also increases the risk. The orientation of stables, buildings, ventilation inlets and outlets, unloading and loading areas, treatment and isolation or quarantine stations should be chosen to minimise the risk of disease introduction and spread (20 - 22). Separate rearing areas for young, sick and new animals with visibly demarcated areas reduce the risk of disease transmission. Natural features such as vegetation, watercourses and topography can benefit a biosecurity plan as they provide natural barriers and drainage opportunities (22, 23).

FEED AND WATER

Feed can be a source of contamination, infection or infestation and can carry or harbour pathogens, chemical residues, weed seeds and pests. Incorrectly stored feed can also spoil, develop unwanted pathogens (e.g. mould) or become contaminated by pests and vermin (11, 22, 23). Important BSMs include purchasing livestock feed from reliable suppliers who pay attention to hygiene and quality, comply with the regulations for feed for feeding ruminants and ensure that it is transported in a clean means of transport (20, 22). It must be ensured that feed is only unloaded after inspection for signs of pests, damage and contamination. It is necessary to check feed for ruminants by taking feed and feed samples from each batch. In addition, ruminant feed must be labelled and stored in such a way that it can be tested at a later date for quality and, if necessary, for the presence of toxins. Store feed in such a way as to avoid contamination from livestock, vermin, wildlife, wild and domestic animals and other feedstuffs. It is extremely important to ensure that ruminants are not fed with products derived from vertebrates, except for tallow and gelatine. When supplying water, care must be taken to ensure that the quantity and quality of the water provided is suitable for the type of ruminant. The quality of drinking water for ruminants should be checked at least once a year by bacteriological analysis. Regular testing of water, soil, feed and fodder is very important when implementing the biosecurity plan at the farm level (22). Water sources are contaminated by faeces or urine and can expose animals to pathogens; they should be cleaned regularly. Manure and dirty water can pose a biohazard as Escherichia coli O157, Salmonella and Campylobacter can survive for up to 3 months (29).

CONTROL PROGRAMS

Control programs are very important elements of biosecurity on ruminant farms (20 - 23). They include written protocols for hygiene procedures, vaccination programs for diseases recommended by a licensed veterinarian (for example in small ruminants - the most common clostridial diseases: Clostridium perfringens types C and D and tetanus, tetanus, caseous lymphadenitis, vibrio and chlamydial abortion, epididymitis and hoof rot), a parasite control program that combines management practices with targeted selective deworming, a rodent control program, an insect control program, an avian control program and

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ensuring controls for the potential spread of disease through carcasses and effluent. Carcasses serve as reservoirs for pathogens, attract pests and are a source for the transmission of pathogens. Various methods of cadaver disposal, including burial, landfilling, incineration, recycling, composting and alkaline hydrolysis, have been reviewed by Nutsch and Kastner (30).

MANAGEMENT PRACTICES

Management practices are also essential elements of biosecurity on ruminant farms (21 - 23). These include a system for identification and traceability of animals, at least annual veterinary inspection of calving, lambing and kidding pens, cleaning and disinfection of materials used for feeding calves/lambs/kids (colostrum, milk, etc.) after birth, separate pens (from maternity and quarantine pens) for sick animals (hospital pens), hospital pens are cleaned and disinfected after each use, aborted animals are tested for non-notifiable diseases, semen is obtained from reputable sources, the application of effluent and manure, particularly of high-risk species, is regulated to minimize the spread of disease through contamination of pasture, feed and water, and vermin, feral animals, weeds and wildlife populations are monitored and regulated to prevent impact on ruminants (20 - 24).

WORKING ROUTINES

Inaccuracies are often made when implementing daily work routines, which are important BSMs. These include the regular inspection of livestock for early detection of sick animals, work routines for farm staff (e.g., the working procedures for farm personnel, e.g., from younger to older animals) should be established, bedding material should be appropriate, wastewater should be collected and contained to prevent access by humans and animals, carcasses and waste should be disposed of as quickly as possible, taking into account environmental and public considerations, disposal areas should be selected to avoid the potential spread of contaminants, disposal areas should be secured and contained to prevent access by livestock, wildlife, domestic animals and wildlife, and finally, regular inspections of properties should be carried out to assess potential biosecurity breaches (20 - 23).

TRAINING, PLANNING AND RECORDING

Planning and recording the implementation of BSMs enables systematic implementation. In this sense, the training of farmers is extremely important. Therefore, a biosecurity plan must be developed for the farm that includes training, planning and recording. Staff should fully understand their role in implementing on-farm biosecurity practices through an introduction to the biosecurity management plan, ongoing biosecurity training and regular review of the on-farm biosecurity protocol. It is of paramount importance that staff responsible for animal husbandry know how to identify sick and injured ruminants and what to do if an animal disease is suspected. Finally, staff should know where to find the contact details of local veterinarians and relevant government officials (20 - 23).

CONSIDERATION OF BIOSECURITY MEASURES

In the literature, one encounters various studies on the use of BSMs in ruminants, such as factors influencing the use of BSMs to protect ruminants and farm workers from infectious diseases (31), the evaluation of biosecurity practices in the production, marketing and slaughter of cattle, sheep and goats (32) and the use of a questionnaire to assess biosecurity in Finnish cattle, pig and sheep farms (26). Various topics were also discussed in connection with cattle, such as biosecurity on cattle farms (25, 33), the basics of biosecurity on cattle farms and good management practices to control infectious diseases (34), the drivers for collective BSMs among cattle and sheep farmers (35), biosecurity standards on cattle farms (36) and a survey on biosecurity and management practices on selected Belgian cattle farms (27). Biosecurity practices on dairy farms have been described in numerous countries such as Brazil (37), Australia (38), Ireland (10) and Canada (39). In addition, biosecurity, health and culling in expanding dairy herds (40) and biosecurity in gastrointestinal diseases of adult dairy cows (12) were considered. Biosecurity and management (13), biosecurity and the management of emergency animal disease (28), biocontainment, biosecurity and security practices (41) and bioexclusion of diseases from dairy and beef farms (15) are topics that have been discussed concerning biosecurity on beef farms.

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Numerous papers point out the importance of BSM implementation at the farm level on different farms in maintaining ruminant health (15, 35, 40 - 45). In addition to the health of ruminants, the implementation of BSMs on ruminant farms is also very important for the prevention of zoonoses (46, 47, 48, 49, 50). The literature emphasizes that most priority bioterrorism agents are zoonotic in origin and that animals could provide an early warning to humans if clinical signs were detected before the onset of human disease or early enough to initiate preventive measures (51).

Studies in the United Kingdom (UK) have shown that farmers and vets have their own relatively clear definitions of biosecurity concerning some key diseases that threaten UK agriculture. Overall, farmers believe that other stakeholders, such as the government, should have more influence on biosecurity in the UK. Conversely, vets saw the ability or willingness of their clients to invest in BSMs as a major barrier. Vets also felt that additional evidence of effectiveness and/or better evidence of the potential economic benefits of proposed on-farm BSMs were needed. Ancillary industries were generally unsure of their role in biosecurity, although study participants highlighted zoonoses as part of the problem and indicated that most barriers exist at the farm level (45).

Studies on farm biosecurity, as perceived by professionals visiting Swedish farms, have revealed many obstacles, especially on sheep and goat farms. Visitors reported that conditions on farms did not allow for an adequate level of biosecurity and that many farmers did not require biosecurity routines, while farmers reported that they expected all visitors to behave professionally and take responsibility for not spreading diseases (52).

Studies on the perceptions and practices of rural veterinarians concerning biosecurity in three European countries revealed different strengths, weaknesses, possible limitations and solutions concerning the veterinary perspective. Veterinarians are seen as key informants by farmers and could therefore play a more active role in advising and improving biosecurity at the farm level. Based on the survey results, two factors appeared to significantly influence the level of implementation of the measures: the country in which the veterinarian practices and the veterinarian's perception of biosecurity. The biosecurity levels with the lowest level of implementation, and therefore posing the greatest risks, were biocontainment and biocontrol (53). It is understood that a sound knowledge of veterinary epidemiology is required to develop disease control programs and implement biosecurity programs at farm, regional and national levels (54).

Farmers have a significant role in the implementation of BSMs. Research on the application of routines that contribute to biosecurity on farms, as reported by Swedish livestock farmers, has shown that a lower level of BSMs is applied on farms with cattle, sheep, goats and mixed species than on pig farms (14). The process of learning about biosecurity and the factors that influence its implementation by farmers is related to the essence of changing human behaviour. To bring about effective change in current biosecurity practices, advisors and farm managers need to understand what motivates and influences workers to use biosecurity (42). Knowledge of the impact of human actions on the risk of infectious diseases (43), opinions and practices of dairy farmers concerning biosecurity and animal welfare (44), determinants of biosecurity behaviour of cattle and sheep farmers in terms of economic analysis (55) are very important aspects.

Various aspects of sheep and goat biosecurity are described in publications on the biosecurity status of small ruminant farms (56), the relationship between structural characteristics and the biosecurity of sheep farms (57) and the implementation of biosecurity in small ruminant farms (58). Different publications further described best management practices for dairy goat farmers (59), biosecurity on sheep farms (20), biosecurity planning guide for goat producers (60), biosecurity for goats (61), biosecurity for sheep and goat producers (24), a national biosecurity planning guide for sheep producers (22) and a national manual for farm biosecurity - grazing animal production (23).

Mixed livestock systems are common in developing countries. A very illustrative example of the application of BSMs in these systems is the study of selected farmers in Australia (38). The results of this study show that 69% of farms operate a mixed livestock system, with the majority of farms keeping either sheep or cattle on the same property as dairy cattle. Around half of the farms (49%) did not provide formal training on animal health aspects to new employees and did not monitor the health of bulls. Most farms (98%) required their employees to wear personal protective equipment such as overalls and boots, but only a few farms (34%) had designated areas for cleaning footwear and a system

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for recording visitors (17%). Most farms kept records of animal health, fence maintenance and the use of supplier declaration forms. The practice of quarantining new animals before bringing them together with other animals was only used on 45% of farms and 55% of farms carried out monthly health visits by a veterinarian.

BSMs are particularly important in "open" herds where ruminants come into contact with animals from other farms (purchase of breeding stock, participation in livestock shows, shared grazing areas, etc.), where more stringent BSMs (i.e. isolation of incoming animals, clinical examinations, laboratory tests, vaccinations, etc.) should be implemented than in closed herds (33).

The risk of introduction and the effect of BSMs varied depending on the type of farm and the transmission route of the disease. Adapting contact patterns to mitigate a particular disease risk was as important as BSMs for some farm types, but the greatest effect was observed when BSMs were combined with better-planned contact patterns. The risk assessment model proved useful for illustrating the risk of endemic disease introduction and the mitigating effect of different BSMs at the farm level (62).

A cross-sectional mixed methods study in Kenya, which included 26 focus group discussions with community members and 10 observational interviews with abattoir staff, a household survey with 560 community members and a separate survey of 231 livestock traders, found that producers, traders and abattoir staff followed some biosecurity practices but not others. The study concluded that implementing BSMs in rural areas is more complex than the biosecurity strategies and frameworks that stipulate them. It also showed that resource constraints, poor implementation and difficult cultural practices can hinder this. Also, the study recommends conducting further studies on the willingness to adopt BSMs targeting community members in resource-poor areas to identify possible critical intervention points at district and state levels (32).

A study of biosecurity practices of cattle herds in Western Canada by Wennekamp et al. (39) found that 54% of herds purchased heifers and 42% purchased cows. The use of standard biosecurity practices was generally low: 30% of producers kept purchased animals separated and 30% of new arrivals were vaccinated. None of the biosecurity practices assessed were associated with the reporting of Johne's disease. The purchase of more than 10 bulls, the purchase of cows, unvaccinated purchased animals and the use of communal pastures were associated with outbreaks of bovine respiratory disease. Outbreaks of calf diarrhoea were associated with the purchase of 10 or more bulls, the use of a communal pasture and the leasing or sharing of bulls.

It is essential to regularly assess the implementation of BSMs using appropriate questionnaires (14, 26, 27, 52, 63). To assess the biosecurity of dairy cattle, beef cattle, veal calves, small ruminants dairy and small ruminants meat, the questionnaires of the University of Ghent can be used (see link: Biochek ugent: https://biocheckgent.com/en/surveys), which can highlight shortcomings that should be addressed immediately. To evaluate the success of the implementation of BSMs, risk assessment is very important as a tool to improve external biosecurity (62). An overview of the methods used to assess biosecurity risk on dairy farms can be found in the paper of Stanković et al. (64), in the Biosecurity Toolkit (1) and in the Guidelines for Import Risk Analysis (65).

CONCLUSIONS

It is very important to implement biosecurity principles and measures as long-standing and successful practices on farms to keep ruminants healthy. These principles and measures should be included in a comprehensive biosecurity plan. A biosecurity plan should address how farmers manage animals, vehicles and human access on the farm, animal health and operations. The most important biosecurity principles should be followed continuously, and in cooperation with government officers and veterinarians, farmers enable themselves to play a significant role in keeping the animals and the production as healthy as possible.

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REFERENCES

1. FAO. Biosecurity Principles and Components. Part. 1. In FAO Biosecurity Toolkit; Food and Agriculture Organization of the United Nations: Rome, Italy, 2007; pp. 1–20. Available at: https://www.fao.org/4/a1140e/a1140e01.pdf (Accessed on: 09-05-2024.).
2. Huber N., Andraud, M., Sassu, E.L., Prigge C., Zoche-Golob V., Käsbohrer A., D'Angelantonio D., Viltrop, A., Zmudzki, J., Jones, H., Smith, R., Tobias, T., Burow E. What is a biosecurity measure? A definition proposal for animal production and linked processing operations. One Health 2022, 15, 100433.
3. Fèvre E. M., Bronsvoort B.M.D.C., Hamilton K.A., Cleaveland S. Animal movements and the spread of infectious diseases. Trends in microbiology 2006, 14(3), 125-131.
4. Lindström T., Sisson S.A., Stenberg Lewerin S., Wennergren U. Estimating animal movement contacts between holdings of different production types. Preventive Veterinary Medicine 2010, 95, 23–31.
5. Nöremark M., Håkansson N., Sternberg-Lewerin S., Lindberg A., Jonsson, A. Network analysis of cattle and pig movements in Sweden: Measures relevant for disease control and risk based surveillance. Preventive Veterinary Medicine 2011, 98, 78–90.
6. Jafari-Gh A., Laven R. A., Eila N., Yadi J., Hatami Z., Soleimani P., Jafari-Gh S., Moazez Lesko M., Heidari E. Transboundary and infectious diseases of small ruminants: Knowledge, attitude, and practice of nomadic and semi-nomadic pastoralists in northern Iran. Small Ruminant Research 2020, 183, 106039.
7. Njenga M.K., Ogolla E., Thumbi S. M., Ngere I., Omulo S., Muturi M., Marwanga D., Bitek A., Bett B., Widdowson M.A., Peninah Munyua Osoro E.M. Comparison of knowledge, attitude, and practices of animal and human brucellosis between nomadic pastoralists and non-pastoralists in Kenya. BMC public health 2020, 20, 1-10.
8. Robinson S.E., Christley R.M. Exploring the role of auction markets in cattle movements within Great Britain. Preventive Veterinary Medicine 2007, 81, 21–37.
9. Hosie B., Clark S. Sheep flock health security. In practice, 2007, 29(5), 246-254.
10. Sayers R.G., Sayers G.P., Mee J.F., Good M., Bermingham M.L., Grant J., Dillon P.G. Implementing BSMs on dairy farms in Ireland. The Veterinary Journal 2013, 197(2), 259-267.
11. Manuja B.K., Manuja A., Singh R.K. Globalization and livestock biosecurity. Agricultural Research, 2014, 3, 22-31.
12. Wells S.J., Dee S., Godden S. Biosecurity of gastrointestinal diseases of adult dairy cattle. Veterinary Clinics of North America: Food Animal Practice, 2002, 18, 35–55.
13. Caldow G. Biosecurity, does it have a place in the management of beef herds in the United Kingdom? Cattle Practice 2004. 12, 149–153.
14. Nöremark M., Frössling J., Lewerin, S.S. Application of routines that contribute to on-farm biosecurity as reported by Swedish livestock farmers. Transboundary and emerging diseases 2010, 57(4), 225-236.
15. Mee J.F., Geraghty T., O'Neill R., More S.J. Bioexclusion of diseases from dairy and beef farms: Risks of introducing infectious agents and risk reduction The Veterinary Journal 2012, 194, 143–150.
16. Sherman D.M. The spread of pathogens through trade in small ruminants and their products. Revue Scientifique et Technique-OIE 2011, 30(1), 207.
17. Sazmand A., Alipoor G., Zafari S., Zolhavarieh S.M., Alanazi A.D., Sargison N.D. Assessment of Knowledge, Attitudes and Practices Relating to Parasitic Diseases and Anthelmintic Resistance Among Livestock Farmers in Hamedan, Iran. Frontiers in veterinary science 2020, 7, 584323.
18. Dewulf J., Van Immerseel F. General principles of biosecurity in animal production and veterinary medicine. In Biosecurity in animal production and veterinary medicine. Eds. Dewulf J., Van Immerseel, F. CABI, 2019, pp. 63-76.
19. Dewulf J., Van Immerseel F. Biosecurity in animal production and veterinary medicine. CABI, 2019.
20. Government of India. 2016. General Guidelines for Biosecurity at Central Sheep Breeding Farm, Hisar. Available at: https://www.dof.gov.in/sites/default/files/2019-12/Biosecurity%20guidelines%20for%20sheep%20and%20goat%20.pdf (Accessed on: 09-05-2024.).
21. Canadian Food Inspection Agency. 2011. National farm-level biosecurity planning guide proactive management of animal resources. Available at: http://www.inspection.gc.ca/animals/terrestrial-animals/biosecurity/standards-and-principles/proactive-management/eng/1374175296768/1374176128059?chap=6 (Accessed on: 09-05-2024.).
22. Canadian Food Inspection Agency. 2013. The National Sheep Producer Biosecurity Planning Guide. Available at: https://publications.gc.ca/site/eng/470917/publication.html?wbdisable=false (Accessed on: 09-05-2024.).

23. Animal Health Australia. 2018. National Farm Biosecurity Manual - Grazing Livestock Production. Available at: https://www.farmbiosecurity.com.au/wp-content/uploads/2019/02/National-Farm-Biosecurity-Manual-Grazing-Livestock.pdf (Accessed on: 09-05-2024.).
24. U.S. Department of agriculture. 2024. Biosecurity for Sheep and Goat Producers. Available at: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/sa_animal_disease_information/sheep-goat/biosecurity (Accessed on: 09-05-2024.).
25. Brennan M. L., Christley R.M. Biosecurity on cattle farms: a study in north-west England. PloS one 2012, 7(1), e28139.
26. Sahlström L., Virtanen, T., Kyyrö, J., Lyytikäinen, T. Biosecurity on Finnish cattle, pig and sheep farms–results from a questionnaire. Preventive Veterinary Medicine 2014, 117(1), 59-67.
27. Sarrazin S, Cay AB, Laureyns J, Dewulf J. A survey on biosecurity and management practices in selected Belgian cattle farms. Prev Vet Med. 2014, 117:129–139.
28. Hernández-Jover M, Higgins V, Bryant M, Rast L, McShane C. Biosecurity and the management of emergency animal disease among commercial beef producers in New South Wales and Queensland (Australia). Prev Vet Med. 2016,134:92–102.
29. Nicholson F.A., Groves S.J., Chambers B.J. Pathogen survival during livestock manure storage and following land application. Bioresour Technol 2005, 96:135–143.
30. Nutsch A, Kastner J. Carcass Disposal Options. In: Wiley Handbook of Science and Technology for Homeland Security. Ed J.Voeller. Wiley, New York, 2008, 1959–1969.
31. Msimang V., Rostal M.K., Cordel C., Machalaba C., Tempia S., Bagge W., Burt F.J., Karesh W.B., Paweska J.T., Thompson P.N. Factors affecting the use of BSMs for the protection of ruminant livestock and farm workers against infectious diseases in central South Africa. Transboundary and Emerging Diseases 2022, 69(5), e1899-e1912.
32. Mutua E.N., Bett B.K., Bukachi S.A., Estambale B.A., Nyamongo I.K. From policy to practice: An assessment of biosecurity practices in cattle, sheep and goats production, marketing and slaughter in Baringo County, Kenya. Plos One 2022, 17(4), e0266449.
33. Valergakis G.E., Arsenos G., Oikonomou G. BSMs on cattle farms. Journal of the Hellenic Veterinary Medical Society, 2008, 59(1), 9-22.
34. Buhman M.J., Dewell G., Griffin D. 2000. Biosecurity basics for cattle operations and good management practices (GMP) for controlling infectious diseases. Cooperative Extension, Institute of Agriculture and Natural Resources, University of Nebraska-Lincoln.
35. Heffernan C., Nielsen L., Thomson K., Gunn G. An exploration of the drivers to bio-security collective action among a sample of UK cattle and sheep farmers. Preventive Veterinary Medicine, 2008, 87, 358–372.
36. Stanković B., Hristov S. 2016. Standardi biosigurnosti na farmama goveda. Poglavlje u monografiji: Optimizacija tehnoloških postupaka i zootehničkih resursa na farmama u cilju unapređenja održivosti proizvodnje mleka (Elektronski izvor]: monografija/urednik Vladan Bogdanović. - Beograd: Univerzitet, Poljoprivredni fakultet, 2016 (Beograd: Poljoprivredni fakultet). - 1 elektronski optički disk (CD-ROM); 12 cm, strana 183 - 210, ISBN 978-86-7834-212-7; CIP 637.1(082)(0.034.2); COBISS.SR-ID 227681036.
37. Ferreira J.S., Baccili C.C., Nemoto B.S., Vieira F.K., Sviercoski L.M., Ienk T., Pagno J.T., Gomes V. Biosecurity practices in the dairy farms of southern Brazil. Frontiers in Veterinary Science 2024, 11, 1326688.
38. Aleri J.W., Laurence M. A description of biosecurity practices among selected dairy farmers across Australia. Animal Production Science 2020, 60(14), 1711-1720.
39. Wennekamp T.R., Waldner C.L., Parker S., Windeyer M.C., Larson K., Campbell J.R. Biosecurity practices in western Canadian cow-calf herds and their association with animal health. The Canadian Veterinary Journal 2021, 62(7), 712.
40. Faust M.A., Kinsel M.L., Kirkpatrick M.A. Characterising biosecurity, health, and culling during dairy herd expansions. Journal of Dairy Science 2021, 84, 955–965.
41. Brandt A.W., Sanderson M.W., DeGroot B.D., Thomson D.U., Hollis L.C. Biocontainment, biosecurity, and security practices in beef feedyards. Journal of the American Veterinary Medical Association, 2008, 232, 262–269.
42. Delabbio J., 2006. How farm workers learn to use and practice biosecurity. Journal of Extension, 2006, 44, 6FEA1.
43. Lindahl J.F., Grace D. The consequences of human actions on risks for infectious diseases: a review. Infection ecology & epidemiology 2015, 5(1), 30048.
44. Hoe F.G.H., Ruegg P.L. Opinions and practices of Wisconsin dairy producers about biosecurity and animal well-being. Journal of Dairy Science 2006, 89, 2297–2308.

45. *Gunn G.J., Heffernan C., Hall M., McLeod A., Hovi M. Measuring and comparing constraints to improved biosecurity amongst GB farmers, veterinarians and the auxiliary industries. Preventive Veterinary Medicine 2008, 84, 310–323.*
46. *Ganter M. Zoonotic risks from small ruminants. Veterinary microbiology 2015, 181(1-2), 53-65.*
47. *Obonyo M., Gufu W.B. Knowledge, attitude and practices towards brucellosis among pastoral community in Kenya, 2013. International Journal of Innovative Research and Development 2015, 4(10), 375-84.*
48. *Nyokabi S., Birner R., Bett B., Isuyi L., Grace D., Güttler D., Lindahl J. Informal value chain actors' knowledge and perceptions about zoonotic diseases and biosecurity in Kenya and the importance for food safety and public health. Tropical animal health and production 2018, 50, 509-518.*
49. *Starič J., Hodnik J J., Drusany Starič K., Ježek J., Janev Holcer N., Leppälä J., Rautiainen, R. 2020. Safety culture regarding zoonoses on domestic ruminant farms. Veterinarska stanica 2020, 51(2), 199-206.*
50. *Youssef D. M., Wieland B., Knight G.M., Lines J., Naylor N.R. The effectiveness of biosecurity interventions in reducing the transmission of bacteria from livestock to humans at the farm level: a systematic literature review. Zoonoses and public health 2021, 68(6), 549-562.*
51. *Rabinowitz P., Gordon Z., Chudnov D., Wilcox M., Odofin L., Liu A., Dein J. Animals as sentinels of bioterrorism agents. Emerging Infectious Diseases 2006, 12(4), 647.*
52. *Nöremark M., Sternberg-Lewerin S. On-farm biosecurity as perceived by professionals visiting Swedish farms. Acta Veterinaria Scandinavica 2014, 56(1), 1-11.*
53. *Renault V., Humblet M.F., Moons V., Bosquet G., Gauthier B., Cebrián L. M., Casal J., Saegerman C. Rural veterinarian's perception and practices in terms of biosecurity across three European countries. Transboundary and Emerging Diseases 2018, 65(1), e183-e193.*
54. *Robertson I.D. Disease control, prevention and on-farm biosecurity: the role of veterinary epidemiology. Engineering 2020, 6(1), 20-25.*
55. *Toma L., Stott A.W., Heffernan C., Ringrose S., Gunn G.J. Determinants of biosecurity behaviour of British cattle and sheep farmers - A behavioural economics analysis. Preventive veterinary medicine 2013, 108(4), 321-333.*
56. *Nooraisyah S., Nurulhuda M.O., Mohd-Shaharizan M.S., Azlina-Azma, I.A. Biosecurity Status of Small Ruminant Farms in Peninsular Malaysia. Malaysian Journal of Veterinary Research 2014, 291, 127.*
57. *Şen Ö.T., Durmuş M., Koluman N. Examination of Structural Characteristics and Biosecurity of Sheep Farms in Niğde Province. Turkish Journal of Agriculture-Food Science and Technology 2023, 11(10), 1847-1854.*
58. *Altınçekiç Ş.Ö., Koyuncu M. Applications of biosecurity in small ruminant livestock enterprises. Hayvansal Üretim (Journal of Animal Production), 2015, 56(1), 48-57.*
59. *Hedrich C., Duemler C., Considine D. Best management practices for dairy goat farmers. University of Wisconsin Emerging Agricultural Markets Team-The Wisconsin Dairy Goat Association, USA, 2008.*
60. *Government of Canada. 2013. Biosecurity Planning Guide for Canadian Goat Producers. Available at: https://inspection.canada.ca/animal-health/terrestrial-animals/biosecurity/standards-and-principles/producer-guide-goats/eng/1375213342187/1375213659306?chap=1 (Accessed on: 09-05-2024.).*
61. *U. S. Department Of Agriculture. 2021. Goat Biosecurity. Available at: http://bit.ly/3c3xQjY >, https://goats.extension.org/goat-biosecurity/ (Accessed on: 09-05-2024.).*
62. *Lewerin S.S., Österberg J., Alenius S., Elvander M., Fellström C., Tråvén M.,Wallgren P., Waller K,P., Jacobson, M. 2015. Risk assessment as a tool for improving external biosecurity at farm level. BMC Veterinary Research, 2015, 11, 1-10.*
63. *Emanuelson U., Sjöström K., Fall N. 2018. Biosecurity and animal disease management in organic and conventional Swedish dairy herds: a questionnaire study. Acta veterinaria scandinavica, 2018, 60, 1-7.*
64. *Stanković B., Hristov S., Relić R. 2018. Dairy farm biosecurity risk assessment methodology - a review. Proceedings of International Symposium on Animal Science, November 22nd – 23rd 2018., Faculty of Agriculture, Belgrade-Zemun, Serbia, pp. 223-230.*
65. *OIE. 2023. Import Risk Analysis - Guidelines for Risk Analysis. Terrestrial Animal Health Code.*

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BIOSIGURNOSNE MERE NA FARMAMA PREŽIVARA (PREVOD)

BIOSECURITY MEASURES ON RUMINANT FARMS

Slavča Hristov[a*], Branislav Stanković[a], Jože Starič[b], Dimitar Nakov[c], Jasna Prodanov-Radulović[d], Bojan Milovanović[e], Ilias Chantziaras[f], Alberto Allepuz[g]

[a]Univerzitet u Beogradu - Poljoprivredni fakultet, Beograd-Zemun, Republika Srbija
[b]Veterinarski fakultet, Ljubljana, Slovenija
[c]Poljoprivredni fakultet Univerziteta Goce Delčev – Štip, Štip, Republika Severna Makedonija
[d]Naučni institut za veterinarstvo „Novi Sad“, Novi Sad, Republika Srbija,
[e]Institut za veterinu Srbije, Beograd, Republika Srbija
[f]Jedinica za veterinarsku epidemiologiju, Fakultet veterinarske medicine, Univerzitet u Gentu, Belgija
[g]Odsek za zdravlje i anatomiju životinja, Autonomni univerzitet u Barseloni, Španija
*Corresponding author: hristovs@agrif.bg.ac.rs

SAŽETAK

U brojnim naučnim radovima, termin biosigurnosne mere (BSM) se definiše kao implementacija segregacije, sanitarnih ili upravljačkih procedura ciljano dizajniranih da smanje verovatnoću unošenja, uspostavljanja, preživljavanja ili širenja potencijalnog patogena u farmu, unutar ili iz farme ili geografsko područje. U radu su prikazane glavne mere biosigurnosti (opšte mere eksterne i interne biosigurnosti koje se odnose na novouvedene životinje, radnike na farmi, članove porodice, posetioce i pružaoce usluga, vozila, alate i opremu, lokaciju farme, vodu i hranu za životinje, programe kontrole, prakse upravljanja, rukovanje sirovinama, radne postupke, obuku, planove i evidenciju) na osnovu podataka iz literature, vodiča, uputstava, preporuka i kontrolnih lista. Pored pomenutih mera biosigurnosti, istaknuti su značaj segregacije, čišćenja i dezinfekcije. Najvažniji i najefikasniji deo biosigurnosti je da se zaražene životinje i kontaminirani materijal drže dalje od neinficiranih životinja. Čišćenje i dezinfekcija štala, vozila i opreme (uključujući čizme i odeću) je veoma efikasan način da se minimizira prenošenje bolesti na ili između životinja. Veoma je mere biosigurnosti važno primeniti kao dugoročnu i uspešnu praksu na farmama za očuvanje zdravlja životinja. Ove mere treba da budu uključene u sveobuhvatni plan biosigurnosti koji se mora u potpunosti primeniti. Planovi biosigurnosti i dizajn i implementacija programa biosigurnosti treba da se pozabave načinom na koji farmeri postupaju sa životinjama, vozilima i pristupom ljudi farmi, kao i zdravljem životinja i radnim procedurama. Ključne mere biosigurnosti treba redovno pratiti i, radeći sa veterinarima, sami farmeri mogu da igraju važnu ulogu u održavanju zdravlja životinja i proizvodnje što je moguće zdravijim. Važno je redovno procenjivati sprovođenje mera biosigurnosti korišćenjem odgovarajućih upitnika, koji mogu da istaknu nedostatke koje treba odmah ukloniti.

Ključne reči: preživari, mere biosigurnosti, plan i program biosigurnosti, upitnici

Uvod

Danas je biosigurnost jasno definisana i smatra se centralnim delom koncepta jednog zdravlja. Međutim, iako se termin biosigurnosne mere (BSM) koristi godinama, još uvek ne postoji konzistentna definicija. Prema Organizaciji Ujedinjenih nacija za hranu i poljoprivredu "biosigurnost je strateški i integrisani koncept koji obuhvata politike i regulatorne okvire (uključujući sredstva i aktivnosti) koji se koriste za analizu i upravljanje rizicima u oblastima bezbednosti hrane, javnog zdravlja, života i zdravlja životinja i biljaka, uključujući povezane rizike po životnu sredinu." (1).

Uzimajući u obzir da nejasna definicija BSM, posebno na nivou farme i politika, može dovesti do nesporazuma, slabog prihvatanja, loše implementacije i samim tim neoptimalne biosigurnosti u lancu proizvodnje hrane, Huber et al. (2) je definisao BSM kao implementaciju postupaka segregacije, sanitacije ili upravljanja koji su posebno usmereni na smanjenje verovatnoće unošenja, uspostavljanja, opstanka ili širenja potencijalnih

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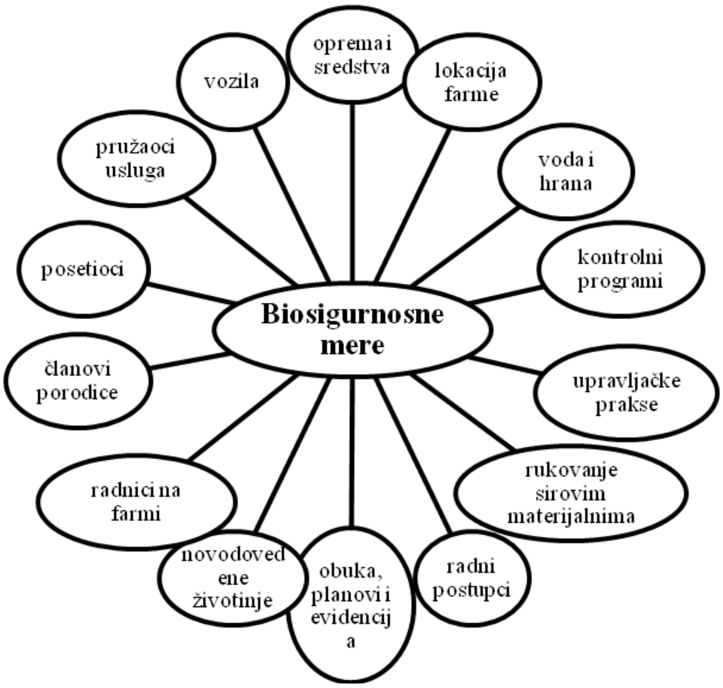
patogena u, unutar ili sa farme ili geografskog područja.
Cilj ovog rada je da se terenski veterinari i farmerii upoznaju sa najvažnijim merama biosigurnosti, odnosno suštinski najvažnijim opštim eksternim i internim BSM koje se odnose na lokaciju farme, novouvedene životinje, radnike na farmi, članove porodice, posetioce i pružaoce usluga, vozila, alate i opremu, vodu i hranu za životinje, programe kontrole, prakse upravljanja, rukovanja sirovim materijalima, procedure rada, obuku, planove i evidenciju, što bi im sve pomoglo da prodube znanje i unaprede međusobnu saradnju.

Biosigurnosne mere

Na grafikonu 1 dat je prikaz biosigurnosnih mera koje se odnose na novodovedene životinje, radnike na farmi, članove porodice, posetioce i pružaoce usluga, vozila, alate i opremu, lokaciju farme, vodu i hranu za životinje, programe kontrole, prakse upravljanja, rukovanje sirovim materijalima, procedure rada, obuku, planove i evidenciju.



Grafikon 1. Biosigurnosne mere na farmama preživara

Uvođenje, izolacija i kretanje životinja

Za širenje bolesti važno je kretanje populacija domaćih i divljih životinja. Brojni nedavni primeri širenja bolesti su posledica kretanja domaćih ili divljih životinja. Razumevanje obima ovih kretanja i povezanih rizika je fundamentalno za razjašnjavanje epidemiologije bolesti, od kojih neke mogu predstavljati zoonotski rizik. Razmatran je značaj globalne trgovine životinjama i istaknuta je uloga neregulisane trgovine životinjama. Diskutovano je o brojnim ključnim primerima gde su kretanja životinja unela patogene u područja koja su ranije bila bez bolesti. Predlažu se mere zasnovane na pojačanom nadzoru za ublažavanje rizika od unošenja novih patogena (3). Važno je razmotriti kontakte kod kretanja životinja između gazdinstava različitih tipova proizvodnje (4, 5), znanje, stav i praksu nomadskih i polunomadskih stočara (6, 7) i ulogu aukcijskih tržišta u kretanju životinja (8).

Brojna istraživanja su pokazala da uvođenje novih životinja na farmu i kretanje životinja (transport i reintrodukcija životinja) igra važnu ulogu u širenju bolesti (3, 8 - 11), zbog čega je održavanje stada bez nabavke životinja sa drugih farmi jedna od najvažnijih biosigurnsnih mera u prevenciji bolesti (3 -5, 10, 12 - 15). Trgovina malim preživarama i njihovim proizvodima je od velikog značaja za biosigurnost, jer ovce i koze mogu da prenesu zoonotske bolesti, kao što su groznica doline Rift, Krimsko-Kongo hemoragična groznica,

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bruceloza i listerioza (16). Stoga su procedure upravljanja koje se odnose na uvođenje novonabavljenih životinja i kretanje životinja na način da se minimizira rizik od unošenja ili širenja zaraznih bolesti od najveće važnosti.

Pregledni rad Mee et al. (15) razmatra dokaze za mnoge puteve prenošenja, kao što je unošenje infektivnih agenasa na farme muznih i tovnih goveda, do kojih može doći njihovim direktnim (kupljena goveda, ponovo uvedena goveda i kontakt sa susednim govedima) ili indirektnim prenošenjem (izmet, posetioci, druge vrste životinja i biološki materijal). U nedostatku programa iskorenjivanja mnogih endemskih zaraznih bolesti, bioisključivanje je najvažnija tehnika upravljanja za smanjenje biosigurnosnog rizika. Najveći rizik za unošenje infektivnih agenasa predstavlja nabavka životinja kada pridošle životinje imaju direktan kontakt sa stadom primaocem u dužem vremenskom periodu (11).

Kako novonabavljene životinje koje ulaze u farmu predstavljaju visok rizik od nenamernog unošenja patogena, neophodno je pre kupovine proveriti njihovo zdravstveno stanje. U prvom redu to znači da životinje treba kupovati samo sa farmi sa istim ili višim zdravstvenim statusom, zatim životinje ne treba kupovati iz stada u kojima se javljaju hromost, apscesi, patološke promene u ustima, lišaji, zamućenja očiju ili drugi klinički znaci bolesti, a takođe životinje namenjene za kupovinu treba pregledati, obraćajući posebnu pažnju na deformisane ili nedavno oštešene rožine papaka. Takođe treba voditi računa o tome da se životinje koje dolaze iz područja gde postoje poznati problemi sa helmintima otpornim na anthelmintska sredstva dehelmintišu i testiraju njihov feces pre dolaska u novo stado kako bi se osiguralo da se u stado ne unesu otporni paraziti (16 - 23).

Potrebno je vršiti segregaciju, posmatranje i po potrebi tretiranje novouvedenih životinja u karantinu, odnosno izolaciju u posebne prostore u zavisnosti od situacije, odvajanje karantinskih objekata od prostora u kome se drži ostatak stada, testiranje životinja na određene bolesti, karantin svih novopridošlih životinja na određeno vreme, a po mogućnosti do posle rođenja. Pored toga, novopridošle životinje moraju biti rutinski dehelminisane i svi testovi na bolesti, tretmani, procedure i vakcinacije moraju biti završeni pre nego što životinje napuste izolaciju. Karantinski padoci ili torovi treba da budu smešteni što je moguće bliže ulazu na farmu ali dalje od drugih životinja. Trebalo bi da postoji dvostruka ograda sa minimalnim rastojanjem od 3 metra između novopridošlih životinja i životinja na farmi. Uopšteno govoreći, farmeri treba da imaju na umu da je najbolje da uzgajaju što više životinja za remont stada na svojoj farmi i da nabavljaju nove životinje sa drugih farmi samo ako je neophodno (10, 18 - 24).

Prilikom transporta životinja na izložbe, sajmove i prodajna mesta, mora se imati u vidu da transportovane životinje mogu preneti uzročnike bolesti na druge životinje mešanjem ili kontaktom sa torovima, vozilima, ljudima i opremom. Da bi se sprečilo prenošenje bolesti na ovaj način, životinje ne smeju da napuštaju gazdinstvo dok za to ne dobiju saglasnost nadležnog veterinara. Takođe se mora obezbediti da sve životinje koje učestvuju na izložbi ili sajmove prođu obavezni veterinarski pregled pre istovara i da se životinje koje pokazuju znake bolesti po povratku ne istovaruju na farmu. Pored toga, treba obezbediti da životinje koje su se nedavno porodile ili su pobacile ili bi mogle da se porode u vreme izložbe budu isključene zbog rizika od prenošenja zaraznih abortivnih bolesti. Životinje treba prevoziti samo u vozilu koje je pre upotrebe očišćeno i dezinfikovano. Važno je izbegavati direktan kontakt sa drugim životinjama i ograničiti blizinu drugih životinja tokom transporta i na izložbama, sajmovima i prodajnim mestima. Farmer mora poneti prostirku i stočnu hranu sa farme i obezbediti snabdevanje čistom vodom na lokacijima izložbi, sajmova i prodajnim mestima i doneti hranilice, kao i posude za vodu i opremu za čišćenje i rukovanje. Rukovanje domaćim životinjama od strane drugih lica mora biti ograničeno. Prilikom prevoza životinja koje se prodaju i preuzimaju sa farme treba voditi računa o dobrobiti životinja u skladu sa propisima o prevozu (18 - 24).

Poznavanje zdravstvenog statusa životinja koje se vraćaju na farmu (npr. životinje koje su učestvovale na izložbama, itd.) je od posebnog značaja za smanjenje rizika od unošenja i širenja bolesti u postojećem stadu. Imajući ovo na umu, mora se obezbediti da životinje koje se vraćaju na farmu budu odvojene na određeni vremenski period od matičnog stada, kako bi se procenila njihova podložnost infekciji. Pri tome treba uzeti u obzir higijenske mere i kontakt sa drugim životinjama tokom odsustva sa farme. Životinje koje se vraćaju na farmu treba zatim testirati u konsultaciji sa veterinarom pre (testovi za određivanje statusa bolesti mogu uključivati serologiju, kulturu i broj

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fekalnih jaja). Životinje treba da ostanu u izolaciji dok rezultati testa ne budu dostupni. Tokom izolacije treba pratiti pojavu kliničkih znakova bolesti i napraviti plan za životinje sa pozitivnim rezultatima testa, koji obuhvata sprovođenje tretmana, zabranu ili odlaganje kupovine, nedeljenje objekata za hranjenje ili pojenje, obora, objekata za rukovanje ili opreme izolovanih životinja osim ako nisu prethodno očišćeni i dezinfikovani. Konačno, svi testovi otkrivanja bolesti, tretmani, procedure i vakcinacije treba da se završe pre nego što životinje napuste izolaciju (18 - 24).

Pri izolaciji i kontroli kretanja, takođe treba uzeti u obzir da farma bude okružena prirodnom barijerom ili ogradom, da je broj pristupnih tačaka farmi minimiziran, da je kretanje ljudi i vozila na i oko imanja svedeno na minimum, da nije dozvoljen kontakt sa životinjama iz drugih stada za vreme ispaše, da se preduzimaju mere za sprečavanje direktnog kontakta između životinja i divljači i na kraju da se preduzimaju mere za sprečavanje direktnog kontakta između preživara i drugih životinjskih vrsta (20 - 23).

Pored toga, treba imati na umu da se odrede "odobrene pristupne zone" za ugovorne strane izvođače na farmi (npr. veterinare, čuvare životinja, osoblja za veštačku oplodnju i dobavljače sena), vozila za isporuku i utovar životnja i sakupljanje proizvoda (npr. cisterne za mleko, kamioni za transport životinja i stočnu hranu) i uslužno osoblje (npr. tehničari za snabdevanje, službenici). Takođe, vozila za isporuku i utovar životnja i sakupljanje proizvoda (npr. cisterne za mleko, kamioni za transport životinja i stočnu hranu) i uslužno osoblje (npr. tehničari za snabdevanje, vladini službenici) treba da budu obeleženi i označeni po potrebi, a takođe bi trebalo da budu objekti obezbeđeni u "odobrenim pristupnim zonama" za izvođače na farmi i posetioce da očiste cipele i opremu po dolasku i pre odlaska sa farme (20 - 23).

U studijama biosigurnosti na farmama goveda u severozapadnoj Engleskoj, postojale su velike razlike između farmi u vrsti i obimu primene biosigurnosnih mera. Većina farmera nije vršila izolovanje kupljenih životinja, dok je mali broj farmera uvek vršio njihovu izolaciju. Mnogi farmeri su sprovodili tretmane nakon selidbe životinja, uglavnom vakcinacije i antihelmintsko tretiranje, ali vrlo mali broj farmera je prijavio da su izvršili zdravstvene preglede. Pored toga, činilo se da postoje velike varijacije u nivou biosigurnosnih mera koje sprovode različite kompanije i izvođači koji su posetili farme. Kompanije za uklanjanje leševa i ugovoreni distributeri životinjskog otpada retko dezinfikuju sebe i svoja vozila, iako postoji velika verovatnoća kontakata sa infektivnim agensima. Ovi nalazi sugerišu da se, iako se sprovode određene prakse biosigurnosti, mnoge od njih retko ili nikada ne koriste. To može biti zbog mnogih faktora, uključujući troškove (vreme i novac), nedostatak dokazane efikasnosti biosigurnosnih praksi i nedostatak obuke veterinara, proizvođača i drugih stručnjaka odgovornih za zdravlje stada. Da bi stočarska proizvodnja u potpunosti koristila preventivnu medicinu, od suštinskog su značaja dalja istraživanja razloga za izostanak upotrebe biosigurnosnih praksi (25).

Radnici na farmi, članovi porodice, posetioci i pružaoci usluga

Osobe koje ulaze na farmu su uglavnom radnici na farmi, članovi porodice, posetioci i pružaoci usluga. Mora se osigurati da njihove aktivnosti i kretanje ne ugrožavaju zdravlje ljudi i životinja. Da bi se to osiguralo, radnici zaposleni na farmi moraju preduzimati mere biosigurnosti. Ovo uključuje osiguranje da svi radnici na farmi znaju i razumiju prakse biosigurnosti na farmi i da su voljni da ih implementiraju i prate promene biosigurnosnih planova i praksi. Pored toga, mora se obezbediti da u slučaju posete pružaoca usluga ili posetilaca, svi zaposleni na farmi budu obavešteni o tome. Brojne studije su pokazale da je ograničen pristup posetiocima biosigurnosna mera koja se često ne primenjuje (25 - 28).

Kretanje vlasnika, zaposlenih lica, posetilaca, veterinara i pružaoca usluga je uobičajeno na farmi i povećava rizik od unošenja i širenja bolesti. Mogu se razviti i primeniti mere za smanjenje ovih rizika. Pristup farmama treba ograničiti uspostavljanjem različitih zona sa različitim nivoima zaštite. Ove zone treba da budu označene granicama i odgovarajućom signalizacijom. Kretanje ljudi u određenu zonu, van određene zone i između određenih zona može se kontrolisati korišćenjem kontrolisanih pristupnih tačaka (22, 23).

Postoji nekoliko važnih principa biosigurnosti koje treba poštovati što je više moguće, a to su: osoblje je isključivo angažovano na farmi (tj. ne deli se sa drugim farmama), posetioci koji ne moraju nužno da dođu u kontakt sa životinjama, kao npr. prodavci, konsultanti, itd., ne

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smeju da imaju kontakt sa životinjama, posetiocima koji apsolutno moraju da dođu u kontakt sa životinjama, kao što su veterinari, osoblje za šišanje, obezivači papaka, itd., obezbeđuju se čizme/prekrivači za cipele i kombinezoni na farmi, dezinfekcione barijere za nogu na ulazu u prostorije, definišu se i postavljaju bisigurnosni znaci gde je to potrebno, obezbeđuje se osmišljen rad na farmi na taj način da minimizira kretanje ljudi, vozila ili opreme u prostore u kojima se drže životinje i vodi se evidencija o posetiocima farme (19).

Svi posetioci i pružaoci usluga moraju biti svesni i pripremljeni za primenu biosigurnosnih mera pre posete. Po ulasku na farmu, svi posetioci i pružaoci usluga moraju da se upišu u registar posetilaca. Nakon registracije, svi oni koji ulaze, rade ili posećuju farmu moraju da operu i dezinfikuju ruke pri ulasku i izlasku sa farme, kada se kreću između pojedinih segmenata i kada ulaze ili izlaze iz određenih rizičnih zona u prostorijama, kao što su prostorije za izolaciju ili tretiranje životinja. Ruke takođe treba oprati ili dezinfikovati pre i posle bilo kakvog kontakta sa životinjama, posebno bolesnim ili životinjama nepoznatog zdravstvenog statusa, kao i nakon kontakta sa potencijalno kontaminiranim materijalima kao što su mrtve životinje, pobačeni fetusi, posteljica ili stajnjak (18, 21 - 24).

Nakon pranja ruku na ulazu, posetioci i pružaoci usluga moraju biti obavešteni o rasporedu prostora na farmi, u kojim prostorima im je dozvoljen pristup i koje biosigurnosne prakse treba da primenjuju na toj lokaciji. Pri tome je potrebno obezbediti da posetioci i pružaoci usluga pristupaju samo delovima farme koji su im neophodni i da im se dozvoli kontakt sa životinjama samo kada je to neophodno. Od velike je važnosti podsticati upotrebu lične zaštitne opreme kada posetioci i pružaoci usluga uđu na imanje farme. Takođe, posetiocima i pružaocima usluga treba objasniti upotrebu lične zaštitne opreme, način odlaganja posle upotrebe (20 - 23).

Pored toga, ne treba zaboraviti sastanke sa radnicima na farmi i članovima njihovih porodica najmanje dva puta godišnje kako bi se razgovaralo o prednostima i efikasnosti svake prakse iz plana biosigurnosti. Na takvim sastancima može se razgovarati o osnovnim temama kao što je napuštanje prostora za životinje bez čišćenja odeće od zagađivača kao što je životinjski izmet ili napuštanje prostora za životinje bez čišćenja i dezinfekcije obuće (19, 22, 23).

Vozila, alati i oprema

Jasno je da bi bilo teško kontrolisati i pratiti širenje bolesti bez ograničenja parkiranja i kretanja vozila unutar površina farme. Biosigurnosne mere predviđaju da vozila koja se kreću od farme do farme mogu da uđu na farmu samo ako su očišćena i dezinfikovana na način da bilo kakva kontaminacija donjih delova ili spoljašnjosti vozila ne može da uđe na farmu. Treba imati u vidu da više neobezbeđenih ulaza na farmu otežava praćenje i kontrolu ulaska vozila na području farme. Stoga, za ulazak u farmu treba koristiti samo jedan ili dva kontrolisana ulaza.

Vozila za prevoz životinja treba da vrše utovaranje i istovaranje na perimetru farme, a životinje se zatim mogu odvesti u zonu izolacije. Vozila za stočnu hranu takođe treba da budu istovarena ili utovarena na određenim mestima, po mogućstvu blizu ispusta za hranu, bez ulaska u glavni deo farme. Treba osigurati da se kamioni koji se koriste za transport stočne hrane ili silaže ne koriste u svrhe koje predstavljaju biosigurnosni rizik za stado i da se pre upotrebe na odgovarajući način očiste i dezinfikuju (20 - 24).

U suštini, kada se prevoze preživari, biraju se samo zdrave životinje koje su u stanju da podnesu utovar kako bi se minimiziralo potencijalno širenje bolesti i/ili kontaminacije putem transporta, transportnim vozilima, hranom, zalihama, sredstvima za održavanje itd. Vozilima je dozvoljen ulazak i napuštanje prostora farme samo ako su propisno dezinfikovani. Transportna vozila za odvoženje životinja i hrane (na klanje, skladišta stočne hrane i sl.) treba da dolaze na farmu prazna (tj. bez drugih životinja), očišćena i dezinfikovana. Posebno treba voditi računa da se uginule životinje i/ili životinjski materijali prikupljaju vozilom za kafileriju i da to vozilo može ukloniti mrtve životinje bez ulaska u prostore farme (20 - 23).

Čišćenje i dezinfekcija je najvažniji alat biosigurnosti za smanjenje rizika od bolesti koje se prenose vozilima. Za vozila koja moraju ući u prostorije farme treba obezbediti prostor za pranje. Ako je moguće, treba koristiti sistem za čišćenje i pranje vozila i opreme pod visokim pritiskom, udaljeno od useva i životinja. Takođe se mora obezbediti da se otpadna voda iz postrojenja za pranje preusmeri sa proizvodnih površina farme. Ako nije moguće obezbediti prostor za pranje, mora se obezbediti da vozila koja ulaze na farmu prolaze kroz postrojenje za pranje točkova, koje je

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postavljeno na ulazu u farmu. Na kraju, važno je voditi evidenciju vozila u koju su uredno upisani svi potrebni podaci prilikom ulaska i izlaska iz farme (20 - 24).

Rizik od širenja bolesti je veći kada se oprema pozajmljuje od drugih farmi, pozajmljuje drugim farmama ili kupuje polovna od drugih farmera. Mora se obezbediti da kretanje i korišćenje opreme i mašina ni na koji način ne ugrožava zdravlje ljudi i životinja, ne samo u okviru farme već i na drugim farmama u blizini. Najbolje je svesti na minimum pozajmljivanje opreme između farmi. Ako se oprema pozajmljuje, treba se pobrinuti da se očisti pre i posle upotrebe. Takođe je potrebno obezbediti specijalne alate, odeću i obuću za određene prostore kao što su proizvodni prostori ili izolacioni prostori u kojima se drže bolesne životinje ili životinje u karantinu. Čišćenje i dezinfekcija igala, alata itd., između svake upotrebe, a posebno između upotrebe u različitim stadima je od najveće važnosti (20 - 22).

Lokacija farme

Rizik od bolesti se višestruko povećava ako se farma nalazi u blizini drugih farmi, klanica, stočnih pijaca, objekata za odlaganje otpada i kafilerija (20 - 22). Lokacija farme u blizini puteva za prevoz životinja i plovnih puteva takođe povećava rizik. Orijentaciju štala, zgrada, ventilacionih ulaznih i izlaznih otvora, prostorija za istovar i utovar, prostorija za tretman i izolaciju ili karantinskih stanica treba birati na taj način da se minimizira rizik od unošenja i širenja bolesti (20 - 22). Odvojeni prostori za uzgoj mladih, bolesnih i novonabavljeni životinja sa vidljivo razgraničenim područjima smanjuju rizik od prenošenja bolesti. Prirodne karakteristike kao što su vegetacija, vodotokovi i topografija mogu biti od koristi u planu biosigurnosti jer pružaju prirodne barijere i mogućnosti drenaže (22, 23).

Hrana i voda

Hrana za životinje može biti izvor kontaminacije, infekcije i može da nosi ili sadrži patogene, hemijske ostatke, seme korova i štetočine. Nepravilno uskladištena hrana se takođe može pokvariti, razviti neželjene patogene (npr. plesni) ili biti kontaminirana štetočinama (11, 22, 23). Važne mere biosigurnosti uključuju nabavku stočne hrane od pouzdanih dobavljača koji vode računa o higijeni i kvalitetu, poštuju propise za hranu koja služi za ishranu preživara i obezbeđuju da se ona transportuje čistim prevoznim sredstvima (20, 22). Mora se osigurati da se hrana istovaruje tek nakon inspekcije na prisustvo štetočina, oštećenja i kontaminacije. Potrebno je proveriti hranu za preživare uzimanjem uzoraka hrane iz svake dospele serije. Pored toga, hrana za preživare mora biti obeležena i uskladištena na način da se kasnije može ispitati na kvalitet i, ako je potrebno, na prisustvo toksina. Treba skladištiti stočnu hranu na način da se izbegne kontaminacija od štetočina, divljih životinja i domaćih životinja i druge hrane za životinje. Izuzetno je važno obezbediti da se preživari ne hrane proizvodima dobijenim od kičmenjaka, sa izuzetkom loja i želatina. Prilikom snabdevanja vodom mora se voditi računa o tome da njena količina i kvalitet odgovara vrsti preživara. Kvalitet vode za piće za preživare treba da se proverava najmanje jednom godišnje bakteriološkom analizom. Redovno ispitivanje vode, zemljišta, stočne hrane i silaže je veoma važno kada se sprovodi plan biosigurnosti na nivou farme (22). Izvori vode koji su kontaminirani izmetom ili urinom mogu izložiti životinje patogenima zbog čega ih treba ih redovno čistiti. Stajnjak i nečista voda mogu dugo predstavljati biosigurnosni opasnost jer Escherichia coli O157, Salmonella i Campilobacter mogu da prežive u njima do 3 meseca (29).

Kontrolni program

Programi kontrole su veoma značajni elementi biosigurnosti na farmama preživara (20 - 23). Oni uključuju pisane protokole za higijenske procedure, programe vakcinacije za bolesti koje preporučuje licencirani veterinar (najčešće protiv klostridijalnih bolesti: *Clostridium perfringens* tipovi C i D i tetanusa, tetanusa, kazeoznog limfadenitisa, vibrio i hlamidijalnog abortusa, epididimiitisa i zarazne šepavosti), programe kontrole parazita koji kombinuju prakse upravljanja sa ciljanom selektivnom dehelmintizacijom, programe kontrole glodara, programe kontrole insekata, programe kontrole ptica, kao i obezbeđivanje kontrole za potencijalno širenje bolesti preko leševa i otpadnih voda. Leševi služe kao rezervoari za patogene, privlače štetočine i izvor su za prenošenje patogena. Različite metode odlaganja leševa, uključujući zakopavanje, deponovanje, spaljivanje, reciklažu, kompostiranje i alkalnu hidrolizu, pregledno su prikazani od strane su (30).

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Prakse upravljanja

Prakse upravljanja su takođe suštinski elementi biosigurnosti na farmama preživara (21 - 23). One uključuju sistem za identifikaciju i sledljivost životinja, veterinarsku inspekciju prostora za teljenje, jagnjenje i jarenje - najmanje godišnje jednom, čišćenje i dezinfekciju materijala koji se koriste za ishranu teladi/jagnjadi/jaradi (kolostrum, mleko, itd.) nakon rođenja, odvojene prostore od porodilišta i karantina za bolesne životinje (prostori za tretman) koji se obavezno čiste i dezinfikuju nakon svake upotrebe. Pored toga, životinje koje su abortirale se testiraju na bolesti koje se ne prijavljuju, sperma treba da se nabavlja iz renomiranih izvora, primena otpadnih voda i stajnjaka, posebno visokorizičnih vrsta, treba da bude regulisana na način da bi se minimiziralo širenje bolesti kroz kontaminaciju pašnjaka, hrane i vode, a štetočine, divlje životinje, korov i populacije divljih životinja treba da se prate i regulišu kako bi se sprečio uticaj na preživare (20 - 24).

Radne rutine

Često se dešavaju nepreciznosti prilikom sprovođenja svakodnevnih radnih rutina, koje predstvaljaju važne biosigurnosne mere. To uključuje redovnu inspekciju životinja radi ranog otkrivanja bolesnih životinja, radne rutine za osoblje farme (npr. treba uspostaviti radne procedure za kretanje osoblja na farmi od mlađih ka starijim životinjama), zatim materijal za prostirku treba da bude odgovarajući, otpadne vode treba da bidu sakupljane i zadržane na način koji bi omogućio sprečavanje pristupa ljudi i životinja, leševi i otpadne materije treba da budu odloženi što je pre moguće, uzimajući u obzir ekološke i javne aspekte, mesta za odlaganje treba da budu odabrana na način da se izbegne potencijalno širenje zagađivača, mesta za odlaganje treba da budu obezbeđena na taj način da bi se sprečio pristup domaćim i divljim životinjama, i na kraju, trebalo bi sprovoditi redovne inspekcije farme kako bi se procenila potencijalna kršenja biosigurnosti (20 - 23).

Obuka, planiranje i evidencija

Planiranje i evidentiranje sprovođenja biosigurnosnih mera omogućava njihovu sistematsku primenu. U tom smislu, obuka farmera je izuzetno važna. Zbog toga se za farmu mora izraditi plan biosigurnosti koji uključuje obuku, planiranje i evidenciju. Osoblje treba u potpunosti da razume svoju ulogu u primeni biosigurnosnih praksi na farmi kroz uvod u plan upravljanja biosigurnošću, stalnu obuku o biosigurnosti i redovnu reviziju protokola o biosigurnosti na farmi. Od izuzetne je važnosti da osoblje zaduženo za stočarsku proizvodnju zna kako da identifikuje bolesne i povređene preživare i šta da radi kada se sumnja na bolest životinja. Konačno, osoblje treba da zna gde da pronađe kontaktne podatke lokalnih veterinara i relevantnih vladinih zvaničnika (20 - 23).

Razmatranje biosigurnosnih mera

U literaturi se susreću različite studije o upotrebi biosigurnosnih mera kod preživara, kao što su faktori koji utiču na njihovo korišćenje za zaštitu preživara i radnika na farmama od zaraznih bolesti (31), evaluacija biosigurnosnih praksi u proizvodnji, marketingu i za vreme klanja goveda, ovaca i koza (32) i korišćenje upitnika za procenu biosigurnosti u finskim farmama goveda, svinja i ovaca (26). Razmatrane su brojne teme u vezi sa biosigurnošću goveda, kao što su: biosigurnost na farmama goveda (25, 33), osnove biosigurnosti na farmama goveda i dobre prakse upravljanja za suzbijanje zaraznih bolesti (34), pokretači zajedničkih biosigurnosnih mera kod goveda i ovaca (35), standardi biosigurnosti na farmama goveda (36), biosigurnost i prakse upravljanja na odabranim belgijskim farmama goveda (27). Prakse biosigurnosti na farmama muznih krava opisane su u brojnim zemljama kao što su Brazil (37), Australija (38), Irska (10) i Kanada (39). Pored toga, razmotreni su aspekti biosigurnosti, zdravlja i uklanjanja grla u rastućim mlečnim stadima (40), kao i biosigurnost u vezi sa gastrointestinalnim bolestima kod muznih krava (12). Biosigurnost i upravljanje (13), zatim biosigurnost i upravljanje kod izbijanja bolesti životinja (28), bioekskluzija, biosigurnost i bezbednosne prakse (41) i bioisključivanje bolesti na farmama muznih i tovnih goveda (15) su teme koje su često razmatrane u vezi sa biosigurnošću na farmama pomenutih preživara.

Brojni radovi ukazuju na značaj primene BSM na nivou farme u očuvanju zdravlja preživara na različitim farmama (15, 35, 40 - 45). Pored zdravlja preživara, primena BSM na farmama preživara je veoma važna za prevenciju zoonoza (46, 47, 48, 49, 50). U literaturi se naglašava da je većina

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agenasa bioterorizma zoonotskog porekla i da bi životinje mogle da posluže kao rano upozorenje ljudima ako se klinički znaci kod njih otkriju pre početka pojave bolesti kod ljudi ili dovoljno rano da se započnu preventivne mere (51).

Studije u Ujedinjenom Kraljevstvu (UK) pokazale su da farmeri i veterinari imaju svoje relativno jasne definicije biosigurnosti u odnosu na neke ključne bolesti koje prete stočarstvu UK. Farmeri smatraju da bi drugi akteri, kao što je vlada, trebalo da imaju veći uticaj na biosigurnost u Velikoj Britaniji. Nasuprot tome, veterinari su kao glavnu prepreku videli sposobnost ili spremnost svojih klijenata da ulažu u biosigurnosne mere. Veterinari su takođe smatrali da su potrebni dodatni dokazi o efikasnosti i/ili bolji dokazi o potencijalnim ekonomskim koristima predloženih biosigurnosnih mera na farmi. Pomoćne industrije generalno nisu bile sigurne u svoju ulogu u biosigurnosti, iako su učesnici studije istakli zoonoze kao deo problema i ukazali da većina prepreka postoji na nivou farme (45).

Studije o biosigurnosti farme, kako ih vide profesionalci koji posećuju švedske farme, otkrile su mnoge prepreke, posebno na farmama ovaca i koza. U osnovi, posetioci smatraju da uslovi na farmama ne dozvoljavaju adekvatan nivo biosigurnosti i da mnogi farmer ne primenjuju biosigurnosne mere, dok farmeri očekuju da se svi posetioci ponašaju profesionalno i da preuzmu odgovornost za neširenje bolesti (52).

Studije o percepciji i praksi veterinara u ruralnim područjima u vezi sa biosigurnošću u tri evropske zemlje otkrile su različite prednosti, slabosti, moguća ograničenja i rešenja u pogledu veterinarske perspektive biosigurnosti. Farmeri smatraju veterinare ključnim osobama koje pružaju informacije, stoga bi oni mogli igrati aktivniju ulogu u savetovanju i poboljšanju biosigurnosti na nivou farme. Na osnovu rezultata istraživanja, pokazalo se da dva faktora značajno utiču na nivo sprovođenja mera: zemlja u kojoj veterinar radi i percepcija veterinara o biosigurnosti. Nivoi biosigurnosti sa najnižim nivoom implementacije, a samim tim i najvećim rizikom, bili su biosigurnosno ograničenje i biosigurnosna kontrola (53). Podrazumeva se da je potrebno dobro poznavanje veterinarske epidemiologije da bi se razvili programi kontrole bolesti i sproveli programi biosigurnosti na farmama na regionalnom i nacionalnom nivou (54).

Farmeri imaju značajnu ulogu u sprovođenju biosigurnosnih mera. Istraživanja o primeni rutina koje doprinose biosigurnosti na farmama pokazala su da se na farmama goveda, ovaca, koza i mešanih vrsta primenjuju niži nivoi mera biosigurnosti nego na farmama svinja (14). Proces učenja o biosigurnosti i faktorima koji utiču na njegovu primenu od strane farmera vezan je za suštinu promene ljudskog ponašanja. Da bi se došlo do efikasne promene u trenutnim biosigurnosnim praksama, savetnici i menadžeri farmi moraju da razumeju šta motiviše i utiče na radnike da primenjuju biosigurnost (42). Poznavanje uticaja ljudskog delovanja na rizik od zaraznih bolesti (43), mišljenja i prakse farmera u vezi sa biosigurnošću i dobrobiti životinja (44), determinantama biosigurnosnog ponašanja farmera u smislu ekonomske analize (55).) su veoma značajni aspekti.

Različiti aspekti biosigurnosti ovaca i koza opisani su u publikacijama o biosigurnosnom statusu farmi malih preživara (56), odnosu između strukturnih karakteristika i biosigurnosti na farmi ovaca (57) i implementaciji biosigurnosti na farmama malih preživara (58). Različite publikacije dalje opisuju najbolje prakse upravljanja za farmere mlečnih koza (59), biosigurnost na farmama ovaca (20), planiranje biosigurnosti za proizvođače koza (60), biosigurnost za koze (61), biosigurnost za proizvođače ovaca i koza (24), planiranje biosigurnosti za proizvođače ovaca (22) i biosigurnost u vezi sa proizvodnjom životinja na ispaši (23).

Mešoviti stočarski sistemi su uobičajeni u zemljama u razvoju. Veoma ilustrativan primer primene biosigurnosnih mera u ovim sistemima je studija koja je obuhvatila farmere u Australiji (38). Rezultati ove studije su pokazali da je 69% farmi ima mešoviti stočarski sistem, pri čemu većina farmi drži ovce i mlečna goveda na istom imanju. Oko polovine farmi (49%) nije obezbedilo formalnu obuku o aspektima zdravlja životinja za novozaposlene i nije pratilo zdravlje bikova. Većina farmi (98%) zahtevala je od svojih zaposlenih lica da nose ličnu zaštitnu opremu kao što su kombinezoni i čizme, ali samo nekoliko farmi (34%) je imalo određene prostore za čišćenje obuće i sistem za evidentiranje posetilaca (17%). Većina farmi je vodila evidenciju o zdravlju životinja, održavanju ograda i korišćenju obrazaca za deklaracije dobavljača. Praksa stavljanja novih životinja u karantin pre njihovog dovođenja u kontakt sa drugim životinjama korišćena je samo na

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45% farmi, a na 55% farmi su obavljane mesečne zdravstvene posete veterinara.

BSM su posebno važni u "otvorenim" stadima u kojima preživari dolaze u kontakt sa životinjama iz drugih farmi (kupovina priplodnih životinja, učešće na stočarskim izložbama, zajedničkim pašnjacima, itd.), gde su strožije mere biosigurnosti (tj. izolacija novonabavljenih životinja, klinički pregledi, laboratorijske pretrage, vakcinacije i sl.) nego u zatvorenim stadima (33).

Rizici od unošenja i efekti biosigurnosnih mera varirali su u zavisnosti od tipa farme i načina prenošenja bolesti. Prilagođavanje obrazaca kontakata da bi se ublažio određeni rizik od bolesti bilo je jednako važno kao i biosigurnosne mere za neke tipove farmi, ali najveći efekat je primećen kada su biosigurnosne mere kombinovane sa bolje planiranim obrascima kontakata. Model procene rizika pokazao se korisnim za ilustraciju rizika od unošenja endemskih bolesti i ublažavanja efekata različitih biosigurnosnih mera na nivou farme (62).

Unakrsna studija mešovitih metoda u Keniji, koja je uključivala 26 diskusija fokusnih grupa sa članovima zajednice i 10 opservacionih intervjua sa osobljem klanice, istraživanje domaćinstava sa 560 članova zajednice i odvojeno istraživanje 231 trgovca životinjama, otkrila je da su proizvođači, trgovci i osoblje klanice sprovodili neke biosigurnosne prakse, ali ne i sve. U studiji je zaključeno da je sprovođenje biosigurnosnih mera u ruralnim područjima složenije od strategija i okvira biosigurnosti koji su propisani. Takođe je pokazala da ograničenje resursa, loša implementacija i kulturne prakse mogu ovo ometati. Preporučuje se sprovođenje daljih studija o spremnosti da se usvoje biosigurnosne mere usmerene na članove zajednice u oblastima sa siromašnim resursima kako bi se identifikovale moguće kritične tačke za intervencije na nivou okruga i države (32).

Studija biosigurnosne prakse stada goveda u zapadnoj Kanadi koju su uradili Vennekamp et al. (39) je otkrila da su kod 54% stada kupovane junice, a kod 42% krave. Upotreba standardnih praksi biosigurnosti je generalno bila niska: 30% proizvođača je držalo kupljene životinje odvojeno, a 30% novonabavljenih životinja je vakcinisano. Nijedna od procenjenih biosigurnosnih praksi nije bila povezana sa prijavljivanjem Joneove bolesti. Nabavka više od 10 bikova, nabavka krava, nevakcinisanih kupljenih životinja i korišćenje zajedničkih pašnjaka povezivani su sa izbijanjem respiratornih bolesti goveda. Izbijanje dijareje kod teladi bilo je povezano sa kupovinom 10 ili više bikova, korišćenja zajedničkih pašnjaka i iznajmljivanja ili deljenja bikova.

Neophodno je redovno procenjivati sprovođenje biosigurnosnih mera korišćenjem odgovarajućih upitnika (14, 26, 27, 52, 63). Za procenu biosigurnosti mlečnih goveda, tovnih junadi, teladi, mlečnih i tovnih malih preživara mogu se koristiti upitnici Univerziteta u Gentu (videti link Biochek ugent: https://biocheckgent.com/en/surveis), koji mogu istaći nedostatke koje treba odmah otkloniti. Za procenu uspešnosti primene biosigurnosnih mera, procena rizika je veoma važna kao alat za poboljšanje eksterne biosigurnosti (62). Pregled metoda koje se koriste za procenu biološkog rizika na farmama mleka nalazi se u radu Stanković i sar. (64), priručniku za biosigurnost (1) i smernicama za analizu rizika kod uvoza (65).

ZAKLJUČAK

Veoma je važno primeniti biosigurnosne principe i mere kao dugogodišnje i uspešne prakse na farmama kako bi preživari bili zdravi. Ovi principi i mere treba da budu uključeni u sveobuhvatni plan biosigurnosti. Plan biosigurnosti treba da se bavi načinom na koji farmeri postupaju sa životinjama, vozilima i pristupom ljudi na farmi, zdravljem životinja i brojnim drugim aktivnostima. Najvažnije principe biosigurnosti treba pratiti kontinuirano, a u saradnji sa državnim službenicima i veterinarima, farmeri omogućavaju sebi da igraju značajnu ulogu u održavanju što je moguće zdravije životinje i proizvodnju.

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A RELATIONSHIP BETWEEN SERUM COPPER CONCENTRATIONS AND HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN SHEEP

VEZA IZMEĐU KONCENTRACIJA BAKRA U SERUMU I HEMATOLOŠKIH I BIOHEMIJSKIH PARAMETARA KOD OVACA

Jožica Ježek*, Jože Starič, Lena Veren Geč, Rok Marzel, Janko Mrkun

Clinic for Reproduction and Large Animals, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, Ljubljana, Slovenia
*Corresponding author: jozica.jezek@vf.uni-lj.si

J.J. https://orcid.org/0000-0002-3753-5944, J.S. https://orcid.org/0000-0002-5156-5277, R.M. https://orcid.org/0009-0000-2753-3374, J.M. https://orcid.org/0000-0002-4439-7367

ABSTRACT

Copper is an essential trace element for animals, required for body, bone and wool growth, pigmentation, nervous system, haemoglobin formation and white blood cell function. The animal's nutrient supply can be assessed with the help of haematological and biochemical blood tests. The aim of the study was to determine the relationship between copper serum concentration and haematological and biochemical parameters in sheep. Sheep from two farms were included in the study. Blood samples were taken from the sheep (40 per flock) twice a year, in the fall after the end of the grazing period and in the spring before the beginning of the grazing period. We carried out 6 consecutive samplings, a total of 480 samples were taken. Haematological (complete blood count) and biochemical analyses of blood samples were performed. The concentrations of copper (Cu), beta-hydroxybutyrate (BHB), total protein (TP), albumin (Alb), urea (Urea), calcium (Ca), inorganic phosphate (iP), magnesium (Mg), sodium (Na), potassium (K) and chlorine (Cl) were measured in the blood serum. The statistical analysis of the results was carried out using the SPSS programme (IBM SPSS Statistics, Ver 29). The comparison (t-test for independent samples) of the investigated parameters for two groups was calculated depending on the Cu concentration in the blood (too low/within the reference values) and the correlation (Pearson) between the Cu concentration and the other investigated parameters. Too low concentration (< 10 µmol/L) of copper was found in 22.3 % of the samples. The mean serum Cu concentration in the low copper group was 7.01 ± 1.84 µmol/L and in the normal copper group 13.73 ± 2.99 µmol/L. Statistically significant lower concentrations of BHB, Ca, urea, Alb, Na, MCV, MCH and WBC were found in the low copper group. We found a statistically significant positive correlation between the concentration of Cu and BHB, urea, TP, Alb, Na, Cl, MCV, WBC, PLT and MCH, and a statistically significant negative correlation between the Cu concentration and RBC. Lower values of the mentioned parameters could be partly related to copper deficiency in sheep. The results show that copper and mineral deficiency could be a problem in sheep grazing on extensive pastures in hilly mountainous regions. In our experience, farmers of small ruminants are often insufficiently aware of the fact that sheep and goats also need mineral and vitamin supplements always available to meet their needs. It would therefore be advisable to pay more attention to educating farmers on this issue. Owners should take care to provide their animals with appropriate mineral mixtures.

Key words: small ruminants, haematology, biochemistry, metabolic profile, blood

SAŽETAK

Bakar je esencijalni element u tragovima za životinje, potreban za rast tela, kostiju i vune, pigmentaciju, nervni sistem, formiranje hemoglobina i funkciju belih krvnih zrnaca. Snabdevanje životinja hranljivim materijama može se proceniti uz pomoć hematoloških i biohemijskih testova krvi. Cilj rada bio je da se utvrdi odnos između koncentracije bakra u serumu i hematoloških i biohemijskih parametara kod ovaca. U istraživanje su uključene ovce sa dve farme. Uzimani su uzorci krvi od ovaca (40 po stadu) dva puta godišnje, u jesen po završetku pašnog perioda i u proleće pre početka pašnog perioda. Uradili smo 6 uzastopnih uzorkovanja, ukupno je uzeto 480 uzoraka. Urađene su hematološke (kompletna krvna slika) i biohemijske analize uzoraka krvi. Koncentracije bakra (Cu), beta-hidroksibutirata (BHB), ukupnog proteina (TP), albumina (Alb), uree (Urea), kalcijuma (Ca), neorganskog fosfata (iP), magnezijuma (Mg), natrijuma (Na), u krvnom serumu su mereni kalijum (K) i hlor (Cl). Statistička analiza rezultata izvršena je korišćenjem SPSS programa (IBM SPSS Statistics, Ver 29). Poređenje (t-test za nezavisne uzorke) ispitivanih parametara za dve grupe izračunato je u zavisnosti od koncentracije Cu u krvi (preniska/u okviru referentnih vrednosti) i korelacije (Pearson) između koncentracije Cu i ostalih ispitivanih vrednosti. Preniska koncentracija (< 10 μmol/L) bakra nađena je u 22,3 % uzoraka. Srednja koncentracija Cu u serumu u grupi sa niskim nivoom bakra bila je 7,01 ± 1,84 μmol/L, au grupi sa normalnom bakrom 13,73 ± 2,99 μmol/L. Statistički značajno niže koncentracije BHB, Ca, uree, Alb, Na, MCV, MCH i VBC utvrđene su u grupi sa niskim sadržajem bakra. Utvrdili smo statistički značajnu pozitivnu korelaciju između koncentracije Cu i BHB, uree, TP, Alb, Na, Cl, MCV, VBC, PLT i MCH, kao i statistički značajna negativna korelacija između koncentracije Cu i eritrocita. Niže vrednosti navedenih parametara mogle bi se delimično dovesti u vezu sa nedostatkom bakra kod ovaca. Rezultati pokazuju da bi nedostatak bakra i minerala mogao biti problem u ispaši ovaca na ekstenzivnim pašnjacima u brdsko-planinskim predelima. Po našem iskustvu, farmeri sitnih preživara često nisu dovoljno svesni činjenice da su ovcama i kozama takođe potrebni mineralni i vitaminski dodaci koji su uvek dostupni da zadovolje njihove potrebe. Stoga bi bilo preporučljivo posvetiti više pažnje edukaciji poljoprivrednika o ovom pitanju. Vlasnici treba da vode računa da svojim životinjama obezbede odgovarajuće mineralne mešavine.

Ključne reči: mali preživari, hematologija, biohemija, metabolički profil, krv

INTRODUCTION

Sheep are frequently reared in semi-extensive, pasture-based farming systems. Sheep farmers are occasionally faced with the problem that production in the flock deteriorates and the expected results are not achieved. The reasons for this usually lie in health problems, nutritional deficiencies and sub-optimal husbandry conditions. Properly managed feeding that ensures an adequate supply of nutrients to the animals is of key importance for successful production and fertility. Inadequate nutrition that does not meet the animals' needs causes them to develop metabolic and deficiency diseases. Deficiency diseases occur most frequently in animals that receive an inadequate or insufficient diet over a long period of time.

Copper is an essential trace element for animals, is a component of many enzymes and is essential for the proper development and function of several organ systems, such as the nervous, hematopoietic, immune, integumentary, skeletal and digestive systems (1). Deficiency in animals can result from a lack of copper in the diet or from an excess of elements (iron, molybdenum, sulphur, calcium carbonate) that reduce copper absorption (2-4). In ruminants, copper has a complex series of interactions with dietary molybdenum and sulphur that, when present in excess, lead to the formation of molybdates and thiomolybdates that bind copper and reduce its absorption and utilization (1). Copper deficiency is most common in animals that eat forage from meadows and pastures on calcareous, soils poor in copper (2, 3). Gastrointestinal parasites may be another cause of hypocuprosis (1). Copper deficiency can lead to anemia, disorders of hair colouring and structure (poor wool quality), neurological problems (ataxia), damage to the heart muscle, increased susceptibility to infection, poor fertility and poor growth. Lambs and lactating ewes are most sensitive to copper deficiency. If the ewes are not supplied with sufficient copper, enzootic ataxia can occur in the lambs. This is a neurodevelopmental disorder characterized by progressive weakness and paralysis, beginning in the hind legs, and inability to suckle (2, 5, 6). Clinical signs are usually not specific enough and are not sufficient to make a reliable diagnosis, so the diagnosis is made by measuring the copper content in the blood serum. In addition to a copper deficiency, an excess is also a problem, as it can

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quickly lead to poisoning, to which sheep are particularly susceptible.
With the help of hematological and biochemical blood tests, we can determine the nutritional status of the animal and the possible presence of diseases (metabolic diseases, inflammations). They make it possible to assess the animal's nutrient supply even before a deficiency becomes clinically apparent. The aim of the study was to determine the relationship between copper serum concentration and hematological and biochemical parameters in sheep.

MATERIAL AND METHODS

Two sheep farms with indigenous breeds (Istrian Pramenka and Jezersko-Solčavska) were included in the study. On both farms, the sheep grazed on hilly pastures in summer, where only extensive cultivation of agricultural land is possible. The winter diet of the sheep differed slightly between the farms. Blood samples were taken from the sheep (40 per flock) twice a year, in autumn and spring. Six samplings were carried out during the project (480 samples in total). The blood samples were taken from the jugular vein in evacuated tubes with EDTA for haematology and in tubes without additives to obtain the blood serum for biochemistry. Haematological analyses (complete blood count) of the blood samples were performed using the Scil Vet abc Plus+ veterinary haematology analyser (Horiba ABX SAS, France). Biochemical analyses were performed using the RX Daytona+ automated biochemistry analyser (Randox Laboratories Ltd, UK). The concentrations of copper (Cu), beta-hydroxybutyrate (BHB), total protein (TP), albumin (Alb), urea (Urea), calcium (Ca), inorganic phosphate (iP), magnesium (Mg), sodium (Na), potassium (K) and chlorine (Cl) were measured in the blood serum.
The statistical analysis of the results was carried out using the SPSS programme (IBM SPSS Statistics, Ver 29). The comparison (t-test for independent samples) of the analysed parameters for two groups was calculated as a function of the Cu concentration in the blood (too low (below 10 µmol/L)/within the reference values) and the correlation (Pearson) between the Cu concentration and the other analysed parameters.

RESULTS

Our results show that farm-related differences in nutrition affect different concentrations of metabolites in the animals' blood. Too low concentration (< 10 µmol/L) of copper was found in 22.3 % of the samples, in 2.5 % from one farm and 41.25 % from another.
The mean serum Cu value in the samples with low copper concentration was 7.01 ± 1.84 µmol/L and in the samples with normal copper 13.73 ± 2.99 µmol/L. We found a statistically significant positive correlation between the concentration of Cu and BHB, urea, TP, Alb, Na, Cl, RBC, MCV, WBC, PLT and MCH. And a statistically significant negative correlation between Cu concentration and RBC (Table 1). Statistically significantly lower concentrations of BHB, Ca, urea, Alb, Na, MCV, MCH and PLT were found in the samples with low copper content compared to the samples with normal copper concentration. The results are shown in Table 2.

DISCUSSION

The low copper content in the samples was attributed to a lack of copper in the sheep's diet and was more common on one farm. The sheep were grazed from spring to autumn, which according to research (2, 3) can be a risk factor for copper deficiency. In part, the lower blood copper concentration could have been influenced by the infestation of the sheep with gastrointestinal parasites, as the increased number of eggs of the gastrointestinal nematodes Strongylida affected the reduced levels of some minerals and also copper. The influence of gastrointestinal parasites on hypocuprosis was also noted by Asin et al. (1). We also found statistically significantly lower levels of Ca and Na in blood samples from sheep with lower copper levels. Gastrointestinal parasites damage the mucosa of the gastrointestinal tract and impair absorption, some suck blood and by their action worsen the supply of nutrients and copper to the animal. Copper deficiency can cause various problems, such as enzootic ataxia in lambs, growth retardation, altered wool quality and depigmentation, anaemia, increased susceptibility to infections (2, 6). In sheep, we found no clinical signs that could be associated with copper deficiency. When analysing the data, we observed statistically significantly lower values of some hematological parameters in low copper samples; MCV and MCH, indicating anaemia. In part, the differences between farms

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could also be influenced by the breed of sheep. Indeed, genetic variation between breeds have also been found in sheep with regard to their copper requirements (6). The breed of sheep in the two flocks studied was different and, in addition to differences in diet, could partly influence the observed differences in copper levels in the samples. A lack of copper is also associated with a weaker immune response. Impaired neutrophil function is one of the earliest functional defects in cattle undergoing copper deficiency, even before clinical signs appear. Neutropenia is a common finding in humans and domestic animals with copper deficiency, in contrast to copper-deficient ruminants in which the number of WBCs does not appear to be impaired (3). In our study, the average number of leucocytes was slightly lower in the samples with lower copper content, but the difference was not statistically significant, which is consistent with the above statement. A lack of copper may also affect the growth of the animals, but we did not find any deviations in our study, the body condition of most animals was adequate. Smaller differences in growth might be detected if the animals would be weighed frequently, which we did not do in the study.

CONCLUSIONS

The proportion of animals with low copper levels differed between farms which is attributed to differences in diet, breed and infestation of the sheep with gastrointestinal parasites.
Using hematological and biochemical blood tests, we can assess the supply of protein, energy and minerals to the sheep. Various metabolic and deficiency diseases can manifest themselves in the animals with similar clinical symptoms, so a blood test is very helpful and often necessary to make the correct diagnosis and determine the cause of the problems. With blood tests in critical phases, when the animal's organism is more stressed (e.g. in periparturition) or when production in the flock is lower than expected, we can recognise possible metabolic or deficiency diseases already in the subclinical phase and prevent an exacerbation of the disease and associated losses through timely action. We believe that the use of metabolic profiling can improve the health, production and welfare of sheep.

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Table 1. Correlation between copper and haematological and biochemical parameters in sheep

Parameter	Cu	
	r	p-value
RBC	-0.130	0.005
Hb	-0.082	0.075
MCV	0.131	0.004
Ht	-0.066	0.152
WBC	0.102	0.026
PLT	0.150	0.001
MCH	0.116	0.011
MCHC	-0.017	0.719
BHB	0.132	0.004
Ca	0.001	0.980
iP	0.072	0.115
Mg	0.017	0.708
Urea	0.255	<0.001
Protein	0.114	0.012
Albumin	0.205	<0.001
Na	0.134	0.003
K	-0.082	0.071
Cl	0.140	0.002

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Table 2. Results of hematological and biochemical parameters (mean, SD) in sheep with low- and normal blood copper concentration

Parameter	Normal Cu	Low Cu	p-value
RBC (10^{12}/L)	10.06±1.48	10.18±1.66	0.482
Hb (g/dL)	10.52±1.34	10.41±1.56	0.454
MCV (fL)	33.38±2.90	32.34±1.75	<0.001
Ht (%)	33.41±4.43	32.83±5.09	0.259
WBC (10^9/L)	8.35±2.52	7.82±2.27	0.056
PLT (10^9/L)	395.03±176.63	352.54±175.18	0.030
MCH (pg)	10.53±0.87	10.27±0.56	0.004
MCHC (g/dL)	31.63±2.42	31.80±2.08	0.522
BHB (mmol/L)	0.42±0.14	0.38±0.10	0.010
Ca (mmol/L)	2.43±0.27	2.34±0.31	0.005
iP (mmol/L)	2.12±0.71	1.98±0.54	0.270
Mg (mmol/L)	0.92±0.12	0.92±0.15	0.917
Urea (mmol/L)	5.84±2.03	4.69±1.93	<0.001
Protein (g/L)	71.14±5.88	70.13±7.99	0.229
Albumin (g/L)	32.90±2.62	30.44±3.28	<0.001
Na (mmol/L)	151.94±3.25	151.10±2.38	0.004
K (mmol/L)	5.06±0.88	5.18±0.69	0,181
Cl (mmol/L)	110.42±4.10	110.20±4.01	0.625

REFERENCES

1. *Asín J., Ramírez G.A., Navarro M.A., Nyaoke A.C., Henderson E.E., Mendonça F.S., Molín, J., Uzal F.A. Nutritional Wasting Disorders in Sheep. Animals 2021, 11, 501. https://doi.org/10.3390/ani11020501*
2. *Gurung N.K., Rush J., Pugh D.G. Feeding and nutrition. In: Sheep, Goat and Cervid Medicine. Ed. D.G. Pugh, A.N. Baird, M. Edmondson, T. Passler. Elsevier, Edinburgh, 2021, pp 15-43.*
3. *Minatel L., Carfagnini J.C. Copper deficiency and immune response in ruminants. Nutrition Research 2000, 20 (10), 1519-1529.*
4. *Jazbec I. Klinično laboratorijska diagnostika. Veterinarska fakulteta, Ljubljana, 1990.*
5. *Scott P.R. Sheep medicine. Manson Publishing Ltd, London,2010.*
6. *Suttle N.F. Mineral nutrition of livestock. 4th ed. CABI, Oxfordshire, 2010.*

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Article
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IN VITRO STABILNOST HORMONA U UZORCIMA KRVI KRAVA

IN VITRO STABILITY OF HORMONES IN COW BLOOD SAMPLES

Danijel Kovačević[a], Dražen Kovačević[a], Vanja Kovačević[a], Mira Majkić[b], Radojica Djoković[c], Marko Cincović[b]

[a]Veterinarska ambulanta Kovačević, Teslić, R.Srpska, BiH
[b]Departman za veterinarsku medicinu, Poljoprivredni fakultet Novi Sad, Srbija
[c]Agronomski fakultet Čačak, Srbija
Autor za korespodenciju: kovacevic.danijel8@gmail.com

R.Dj. https://orcid.org/0000-0003-0900-3227, M.C. https://orcid.org/0000-0002-5683-8983, M.M. https://orcid.org/0009-0007-0110-7327

SAŽETAK

Hormoni su veoma značajni kod krava jer učestvuju u orkestriranom prestrojavanju organizma na laktaciju koja dolazi (homeoreza) i kao takvi imaju veliki dijagnostički i prognostički značaj, pa je održavanje njihove stabilnosti u uzorku od presudne važnosti. U ovom istraživanju je učestvovalo 300 uzoraka poreklom od mlečnih krava u različitim periodima laktacija. Uzorci su podeljeni u 6 grupa: prema vremenu od uzorkovanja do obrade u laboratoriji (0-4h, 4-8 h i preko 8h) i prema prisustvu preanalitičkih faktora (grupa sa hemolizom, grupa transportovana na ambijentalnoj temeperaturi i grupa sa malom zapreminom uzorka). Uzorci su ostavljani na +4°C i testirani su jednom dnevno tokom 6 dana čuvanja uzorka. Uzorci koji su stavljeni na -20°C testirani su jednom mesečno tokom 6 meseci. Maksimalno dozvoljena nestabilnost izračunata na osnovu analitičke i intraindividualne varijabilnosti kretala se od 6,2 do 6,8 % (T3, T4, STH. IGF-I i progesteron) odnosno do 7,1 do 7,2 % (za insulin i kortizol) i smatra se da je uzorak izgubio stabilnost ako se tokom veremena desi promena vrednosti parametara u procentu iznad ove maksimalno određene vrednosti. Najmanju stabilnost na +4°C pokazao je progesteron koji se može čuvati do 48h. Sledeći je bio insulin koji može biti stabilan do 3 dana u uzorku koji je opterećen preanalitičkim greškama, ali je bio stabilniji u uzorcima koji nisu bili opterećeni preanalitičkim grešakam. Kortizol, T3 i T4 imaju manju stabilnost pa se mogu čuvati do 4 ili 5 dana ako je uzorak opterećen preanalitičkim greškama. Kod zamrznutih uzoraka nije nađeno značajnije opadanje stabilnosti tokom eksperimentalnog perioda. Na stabilnost endokrinoloških parametara utiču osobine hormona i postojanje preanalitičkih grešaka u epruveti.

Ključne reči: krave, endokrini parametri, maksimalno dozvoljena nestabilnost, preanalitičke greške.

ABSTRACT

Hormones are very important in cows because they participate in the orchestrated rearrangement of the organism for the coming lactation (homeoresis) and as such have great diagnostic and prognostic significance, so maintaining their stability in the sample is of crucial importance. Three hundred samples originating from dairy cows in different periods of lactation participated in this research. The samples were divided into 6 groups: according to the time from sampling to processing in the laboratory (0-4h, 4-8h and over 8h) and according to the presence of pre-analytical factors (group with hemolysis, group transported at ambient temperature and group with small sample volume). The samples were left at +4°C and were tested once a day during 6 days of sample storage. Samples that were placed at -20°C were tested once a month for 6 months. The maximum permissible instability calculated on the basis of analytical and intraindividual variability ranged from 6.2 to 6.8 % (T3, T4, STH. IGF-I and progesterone) and up to 7.1 to 7.2 % (for insulin and cortisol) and it is considered that the sample has lost its stability if during the time there is a change in the value of the parameters in a percentage above this maximum determined value. The lowest stability at +4°C was shown by progesterone, which can be stored for up to 48 hours. Next was insulin, which can be stable for up to 3 days in a sample affected by preanalytical errors, but was more stable in samples not affected by preanalytical errors. Cortisol, T3 and T4 have less stability and can be stored for up to 4 or 5 days if the sample is loaded with pre-analytical errors. With frozen samples, no significant decrease in stability was found during the experimental period. The stability of endocrinological parameters is influenced by the properties of hormones and the existence of pre-analytical errors in the test tube.

Key words: cows, endocrine parameters, maximum permitted instability, preanalytical errors.

HORMONI KOD KRAVA I NJIHOV ZNAČAJ U HOMEOSTAZI I HOMEOREZI

Hormoni su veoma značajni kod krava jer učestvuju u orkestriranom prestrojavanju organizma na laktaciju koja dolazi (homeoreza) i kao takvi imaju veliki dijagnostički i prognostički značaj (1-3), pa je održavanje njihove stabilnosti u uzorku od presudne važnosti.

Insulin je hormon koji se sintetiše u B-ćelijama endokrinog pankreasa i ima vrlo značajnu ulogu u metabolizmu organskih materija, naročito ugljenih hidrata i masti. Posebno je značajna uloga insulina u metabolizmu masti i ugljenih hidrata kod preživara za vreme povećanih metaboličkih zahteva, odnosno u periodima graviditeta i početkom laktacije. Kod krava u laktaciji dolazi do smanjivanja koncentracije insulina u krvi, zbog čega dolazi do usmeravanja prekurzora ka mlečnoj žlezdi i time njihovog većeg iskorišćavanja za sintezu sastojaka mleka. Takođe, ovi autori su utvrdili negativnu korelaciju između koncentracije insulina u krvi i proizvodnje mleka, a značajno pozitivnu sa povećanjem telesne mase. Nađeno je da u uslovima povišene insulinemije postoje sve pogodnosti da se značajan deo prekurzora usmeri za sintezu telesne masti i time umanji nivo aktivnosti mlečne žlezde u pogledu lučenja maksimalne količine mleka, a to je posledica manje osetljivosti mlečne žlezde na delovanje insulina u odnosu na masno tkivo. Utvrđena je značajnu negativnu korelaciju (r =-0.55) između koncentracija insulina i slobodnih masnih kiselina u puerperijumu kod krava i smatraju da snižavanje koncentracije insulina u krvi u puerperijumu omogućava prelazak organizma na masne kiseline kao glavno metaboličko gorivo. Ovaj proces je posebno značajan kod ketoznih krava kod kojih su utvrđene niže vrednosti insulina u odnosu na zdrave krave u puerperijumu i kod kojih se kao posledica toga pojačava lipomobilizacija, a velike količine masnih kiselina u jetri koriste za stvaranje ketonskih tela. Zbog toga je u krvi ketoznih krava pored hipoinsulinemije prisutna hiperlipidemija, hipoglikemija i hiperketonemija.

Trijodtrironin (T_3) i tiroksin (T_4) su hormoni štitaste žlezde. Uloga tireoidnih hormona je veoma značajna kod visoko-mlečnih krava. Utvrđeno je da su kod krava sa visokom mlečnošću značajno niže vrednosti koncentracije trijodtironina i tiroksina u krvi u odnosu na vrednosti kod krava sa niskom mlečnošću ili kod krava u periodu zasušenja sve do teljenja. Nedovoljno unošenje energije, kao i negativni energetski bilans su usko povezani sa smanjivanjem koncentracije tiroksina u krvi, a posebno trijodtironina u peripartalnom periodu kod mlečnih krava. Niski nivoi trijodtironina i tiroksina u krvi tokom laktacije kod krava posledica smanjene aktivnosti tireoidne žlezde, posredstvom hipotalamo-hipofizne osovine uzrokovane stanjem visoke produkcije mleka. Kod mlečnih krava je utvrđeno da su koncentracije trijodtironina i tiroksina u krvi niže kod krava u laktaciji u odnosu na krave kod kojih mlečna žlezda nije aktivna. Takođe, koncentracije trijodtironina i tiroksina su stalno niže kod krava sa većom produkcijom mleka u odnosu na krave sa manjom produkcijom mleka. Razlike su bile statistički značajne i to od 40 do 305 dana laktacije. Smatra se da relativno niske koncentracije trijodtironina i tiroksina u krvi visoko-produktivnih krava, mogu biti posledica razlika u energetskom metabolizmu između visoko-produktivnih i nisko-produktivnih krava.

Kortizol je najznačajniji glukokortikosteroid kod preživara. On ima osnovnu funkciju u regulaciji metabolizma organskih materija. Kod preživara kortizol ima važnu ulogu u regulisanju procesa glukoneogeneze i deponovanju glikogena u jetri, kako kod normalno hranjenih tako i gladnih životinja. Na metabolizam masti kortizol ima pretežno kataboličku ulogu.

Hormon rasta (STH) - Pozitivna korelacija između promena u količini proizvedenog mleka i koncentracije hormona rasta u odnosu na koncentraciju insulina u krvi, ukazuje da hormon

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rasta za razliku od insulina, stimuliše proces lipolize iz telesnih depoa i povećava količinu raspoloživih prekurzora za produkciju mleka.

Faktor rasta sličan insulinu (IGF-I) sintetiše se u jetri pod uticajem somatotropina i ova stimulacija predstavlja temelj osovine STH - IGF-I. Period rane laktacije kod krava karakteriše dugotrajni negativni energetski bilans i za to vreme jetra postaje neosetljiva na delovanje STH, što uzrokuje izraženo smanjenje koncentracije IGF-I u cirkulaciji.

Tokom lutealne faze corpus luteum produkuje velike količine progesterona, pa se koncentracija progesterona u plazmi povećava za dvadeset puta. Progesteron ima kratak poluživot i u jetri se konvertuje u pregnandiol, a on u glukuronsku kiselinu koja se ekskretuje urinom. Ovaj hormon inhibira oslobađanje GnRH iz hipotalamusa i posledično izlučivanje FSH i LH iz adenohipofize. Koncentracija progesterona, koja se povećava u toku lutealne faze, utiče na lučenje LH Progesteron ispoljava antiestrogeni efekat i na miometrijalne ćelije u smislu smanjenja njihove ekscitabilnosti, senzitivnosti na oksitocin, stimulatoran efekat na nivou endometrijuma, ali tako što stimuliše sekretorne ćelije te se time obezbedjuju uslovi za nidaciju oplođene jajne ćelije. Smatra se da su estrogeni odgovorni za tzv. proliferativnu fazu u ciklusu uterusa, a progesteron za sekretornu fazu. Pored toga, progesteron povećava sekretornu aktivnost (lučenje sluzi) jajovoda, što je pored ostalog važno da obezbedi optimalnu sredinu za prolaz spermatozoida. Na nivou mlečnih žlezda progesteron stimuliše razvoj lobulusa i alveola tako što indukuje diferencijaciju već estrogenima pripremljenog duktalnog tkiva i potpomaže funkciju mlečnih žlezda tokom laktacije. Merenje koncentracije progesterona (P4) je do sada najviše korišćeni metod otkrivanja graviditeta kod domaćih životinja. Iako nije specifičan samo za graviditet, progesteron se može koristiti kao test hormon na graviditet jer žuto telo opstaje tokom ranog graviditeta kod svih domaćih životinja.

PREANALITIČKE GREŠKE I STABILNOST UZORAKA

Najveći broj informacija tokom dijagnostičkog odlulčivanja veterinari dobijaju kroz laboratorijske rezultate, a potom kroz kliničku sliku. Zbog toga je laboratoratorijsko testiranje od velikog značaja. Preanalitička faza rada je najveći izvor grešaka i čini oko 70% svih grešaka u laboratoriji (4-9). Faktori koji deluju u ovoj fazi mogu na najrazličitije načine da promene vrednosti laboraorijskih parametara koji se mere, što može dovesti do pogrešne interpretacije dobijenih rezultata, što u krajnjoj liniji može ugroziti i život životinje, odnosno tehnološke procedure na farmi. Preanalitički faktori se mogu kategorisati kao tehnički faktori (koji se odnose na način uzorkovanja i organizacijom postupanja sa uzorcima pre same analize, izbor antikoagulansa, skladištenje, transport i čuvanje uzoraka i očuvanje stabilnosti uzorka do analize) i kao biološki faktori (koji se odnose na svojstva jedinke). Potrebno je u svakoj laboratoriji i za svaku vrstu dobro poznavati tehničke preanalitičke faktore i izvršiti njihovo identifikovanje, kvantifikovanje i kontrolu pomoću stalnih standardnih procedura laboratorije. Biološki faktori čine mnogo šire polje koje se tiče različitih osobina jedinki kao što su starost, zdravstveni status, gladovanje, stres, sedacija, telesna kondicija, reproduktini status i dr., koji su teži za kontrolu, ali je bitno da budu kvalitetno dokumentovani i uzeti u obzir prilikom interpretacije laboratorijskih rezultata. U uslovima rada sa farmskim životinjama, dešava se da ovaj deo preanalitičke faze nije pod kontrolom stručnjaka iz laboratorije, već zavisi od obaveštenosti i obučenosti veterinara sa terena koji šalje uzorke na ispitivanje. Zbog toga je bitno da farmski veterinari budu obučeni za razumevanje kako tehničkih tako i bioloških preanalitičkih faktora, je rod njih u najvećoj meri zavisi odekvatnost uzorka, njegovog transporta kao i dostupnost podataka o jedinki za koju vršimo određivanje metaboličkog profila.

Preanalitički faktori u velikoj meri mogu uticati na vrednosti i stabilnost analita u krvi. Prolongirani kontakt seruma sa ćelijskim komponentama i koagulumom smanjuje stabilnost analita, ali se serum smatra stabilnijim uzorkom u odnosu na plazmu. Upotreba različitih vakutajnera i antikoagulanasa može dovesti do znatnog odstupanja u vrednosti metabolita u plazmi kod mlečnih krava u odnosu na serum (10). Dugotrajno čuvanje uzorka na -20°C daje prihvatljive rezultate tokom 90 dana, dok se prolongirana stabilnost analita postiže prilikom čuvanja na -70°C (11). Ispitivanja pokazuju da preanalitički i postanalitički faktori (vreme transporta uzorka i temperatura čuvanja uzoraka) mogu imati značajan uticaj na varijabilnost parametara u analitičkoj fazi (12). Hemoliza smanjuje stabilnost vitamina, hormona i lekova u

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krvi, a u našem istraživanju se pokazalo da je veliki broj analita manje stabilan u hemoliziranim uzocima (13,14). Nedovoljno punjenje uzorka dovodi do razvoja hemolize i promene u vrednosti metabolita (15). Nedovoljno punjenje nastaje kao posledica loše venepunkcije, slabog vakuma u tubi, ili kao posledica otvaranja vakum tube, što često dovodi mešanja sa vazduhom, kada dolazi do razvoja pene u uzorku, mehaničkog oštećenja uzorka, hemolize i uticaja na analite (16). Pored hemolize u ovakvim uzorcima može biti problem sa izmenjenom turbidimetrijom, verovatno zbog incomplete clot formation i postojanja latent fibrin formation (17). Ostavljanje uzorka na ambijentalnoj temperaturi, posle čega se odlažu na +4°C dovodi do značajnog variranja analita u odnosu na proceduru u kojoj se uzorci odmah stavljaju na +4°C (18), sa čime se slažu naši rezultati.

Utvrđen je nivo uticaja preanalitičkih grešaka na stabilnost velikog broja parametara kod životinja i ljudi (19-28). Koncentracija NEFA na +4°C je bila očuvana samo kod brzog transporta uzorka u laboratoriju, dok prolongirani transport i prisustvo preanalitičkih faktora značajno smanjio stabilnost NEFA na 1-3 dana (24-48h). NEFA je bila stabilna na -20°C tokom celog ispitivanog perioda. Naši rezultati se slažu sa Stokol and Naydan (2005) (29) koji su pokazali da NEFA concentrations were stable at 4°C for 72 h in separated plasma or serum i znatno veću stabilnost na -20°C. BHB je pokazao stabilnost u svim uslovima ispitivanja i svim vremenskim tačkama, što se takođe slaže sa predhodno citiranim autorima. Porast koncentracije NEFA tokom vremena na +4°C nađen je i kod ovaca i ljudi (30,31). Koncentracija Ca je bila stabilna bez obzira na delovanje preanalitičkih faktora, način čuvanja i time-point. Slične rezultate su dobili Bach i sar. (2021) (32). Zbog degradacije uzoraka dolazi do opadanja koncentracije analita tokom vremena. Potencijalni klinički značaj je utvrđen u određenom procentu uzoraka za TBIL, AST, NEFA, BHB i Ca. Potencijalni klinički značaj je potvrđen za TBIL u predhodnom testu stabilnosti (33). Utvrđene su granične vrednosti ovih parametara koje sa visokom specifičnošću i senzitivnošću ukazuju na različite bolesti ili lošu metaboličku adaptaciju (34-36), pa je potrebno razmotriti da li odstupanja vrednosti analita tokom testa stabilnosti može dovesti do pogrešne intepretacije u određenoj vremenskoj tački.

Standardne smernice za rukovanje uzorcima krvi navode da bi plazmu ili serum trebalo odvojiti od ćelija što je pre moguće, a svakako u roku od 2 sata. Iako je to potrebno za određene analite, može se pretpostaviti da se mnogi krvni testovi pogoršaju u roku od nekoliko sati nerazdvojeni uzorci čuvaju se na temperaturi okoline. Ova uočena potreba za trenutnim lokalnim odvajanjem uzoraka krvi ili njihovim brzim hladnim prenosom u centralnu laboratoriju, što povećava složenost i troškove, može spriječiti uključivanje prikupljanja krvi u opsežne epidemiološke studije. Posebno kada se uzorci krvi prikupljaju na velikom broju zasebnih lokacija u okviru studije, slanje uzoraka pune krvi u centralnu laboratoriju za odvajanje može biti prikladnije i isplativije od dogovaranja za lokalno odvajanje ili za kurirski transport ohlađenih uzoraka (37-45).

ISPITIVANJE STABILNOSTI HORMONA

U ovom istraživanju je učestvovalo 300 uzoraka poreklom od mlečnih krava u različitim periodima laktacija. Uzorci su podeljeni u 6 grupa: prema vremenu od uzorkovanja do obrade u laboratoriji (0-4h, 4-8 h i preko 8h) i prema prisustvu preanalitičkih faktora (grupa sa hemolizom, grupa transportovana na ambijentalnoj temeperaturi i grupa sa malom zapreminom uzorka). Uzorci krvi su uzimani iz *v.coccigea* u adekvatnim tubama. Posle centrofugovanja, količina seruma je bila 5-6 mL, osim u uzorcima male zapremine koji su se kretali oko 2-3 mL.

Uzorci su ostavljani na +4°C i testirani su jednom dnevno tokom 6 dana čuvanja uzorka. Uzorci koji su stavljeni na -20°C testirani su jednom mesečno tokom 6 meseci. Gómez-Rioja i sar. (2019) (46) u svom protokolu precizno su definisali tehničke procedure za merenje stabilnosti. Gubitak stabilnosti se procenjuje poređenjem mernih vrednosti dva uzorka dobijena od istog pacijenta i analizirana u različitim vremenskim tačkama – bazalno vreme/uzorak i vreme ispitivanja/uzorak. Razlike su izražene procentualnom devijacijom (PD%). PD% je razlika između izmerenih koncentracija u optimalnim uslovima (osnovni uzorak; t0, prosek ponovljenih merenja) i njegovih koncentracija kada se skladište za maksimalno dozvoljeno vreme skladištenja (uzorak za testiranje; tk prosek ponovljenih merenja). Razlika se pretvara u %PD od osnovne vrednosti: $PD\ \% = ((tk-t0)/t0) \times 100$. MPI je izračunat $MPI = 0,5 \times (CVa+CVi)$. $PD\% < MPI$ za sve ispitanike ukazuje na to da nije došlo do značajnog gubitka stabilnosti unutar proučenog vremena skladištenja. $PD\% \geq MPI$ kod nekih ispitanika ukazuje na to da je došlo do

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značajnog gubitka stabilnosti unutar proučenog vremena skladištenja. Ispitana je i stabilnost endokrinooških parametara u funkciji postojanja preanalitičkih faktora. U prvom koraku je određen maksimalno dozvoljeni nivo nestabilnosti na osnovu predhodno dobijenih varijabilnosti u Laboratoriji za patološku fiziologiju. Vrednosti MPI za odabrane hormone prikazani su u tabeli 1. U grafikonima je prikazana je dinamika promene vrednosti endokrinoloških parametara na temperaturi frižidera i zamrzavanja.

Kada se radi o hormonima najveću osetljivost prema hemolizi je pokazao INS. Značajno opadanje njegove vrednosti je posledica toga što se tokom hemolize iz eritrocita oslobađaju protease koje uništavaju peptidne hormone kao što je INS (47). T3, T4 and CORT pokazuju nižu koncentraciju prilikom dodavanja hemoliziranog uzorka, ali je potrebno da postoji veoma visok nivo hemolize da bi došlo do biasa koji prevazilazi analitički odnosno klinički dozvoljenu varijabilnost ovih hormona. CORT i T4 su hormoni vezani za proteine u krvotoku, pa tačno merenje ovih hormona pre svega zavisi od sposobnosti eseja da molekule hormona odvoji od binding proteina (48). Naše metode podrazumevaju hemiluminiscentni način merenja hormona kod kojih a hemoliza ne pokazuje spektrofotometrijsku interferenciju (49), što potvršđuje da je postojao dominantan uticaj hemijske interferencije na koncentraciju hormona preko proteaza koje degradiraju hormone, antitela i druge proteinske sisteme značajne za tačno određivanje hormona u nekom eseju.

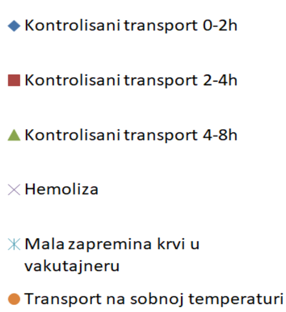
Postoje različiti indeksi koji se koriste za određivanje stabilnosti uzorka. Najčešće se koriste sledeći (izrazi su dati na engleskom jeziku zbog sledljivosti): critical change value (CCV), the maximum permissible instability (MPI) and the total allowable error (TEa). MPI je mnogo striktniji kriterijum, jer dovodi do odbacivanja većeg broja analita u određenoj vremenskoj tački u odnosu na CCV, koji znatno veći broj analita čini prihvatljivim (50).

Prolongirani transport u idealnim uslovima ili postojanje preanalitičkih faktora kao što su neadekvatan transport na sobnoj temperaturi, hemoliza ili mala zapremina uzorka skraćuju stabilnost uzoraka u oba temperaturna režima. Ova studija može pomoći u definisanju prihvatljivog vremena transporta i čuvanja za uzorke krvi goveda, što je od velikog značaja, jer u radu sa farmskim životinjama često nije moguće u kratkom roku uzeti uzorke i dopremiti ih u laboratoriju, a uzorci su često opterećeni određenim preanalitičkim faktorima uz ograničene mogućnosti ponavljanja uzorkovanja. Smanjenje vremena transporta od farme do laboratorije je ključna stvar, jer prolongirani transport u kombinaciji sa postojanjem drugih preanalitičkih faktora u mnogome smanjuje stabilnost uzoraka, pa je potrebno što ranije izvršiti analize. Stabilnost krvnih parametara i interferencija sa preanalitičkim faktorima pokazuje određene specifičnosti za vrstu, pa se ne mogu u potpunosti preuzeti parametri stabilnosti koji su određeni zadruge vrste životinja ili ljudi, što potvrđuje značaj izvedenog istraživanja na mlečnim kravama. U našim ranijim istraživanjima potvrdili smo značaj preanalitičkih faktora u oceni stabilnosti biohemijskih parametara krvi kod krava (51), pa zaključujemo da poznavanje stabilnosti i dinamičke promene metabolita i hormona tokom vremena može značajno da utiče bias ovih parametara, a posledično i na interpretaciju rezultata, pa je potrebno poznavati stabilnost tokom izrade endokrinog i metaboličkog profila u rutinskoj praksi.

Tabela 1. Maksimalno dozvoljena nestabilnost (MPI) endokrinoloških parametara u krvi krava

Biohemijski parametar	Maksimalno dozvoljena nestabilnost (MPI)
INS	7,1
CORT	7,2
T3	6,1
T4	6,3
IGF-I	6,2
STH	6,8
PROG	6,4

Legenda:



Maksimalno dozvoljena nestabilnost izračunata na osnovu analitičke i intraindividualne varijabilnosti kretala se od 6,2 do 6,8 % (T3, T4, STH. IGF-I i progesteron) odnosno do 7,1 do 7,2 % (za insulin i kortizol) i smatra se da je uzorak

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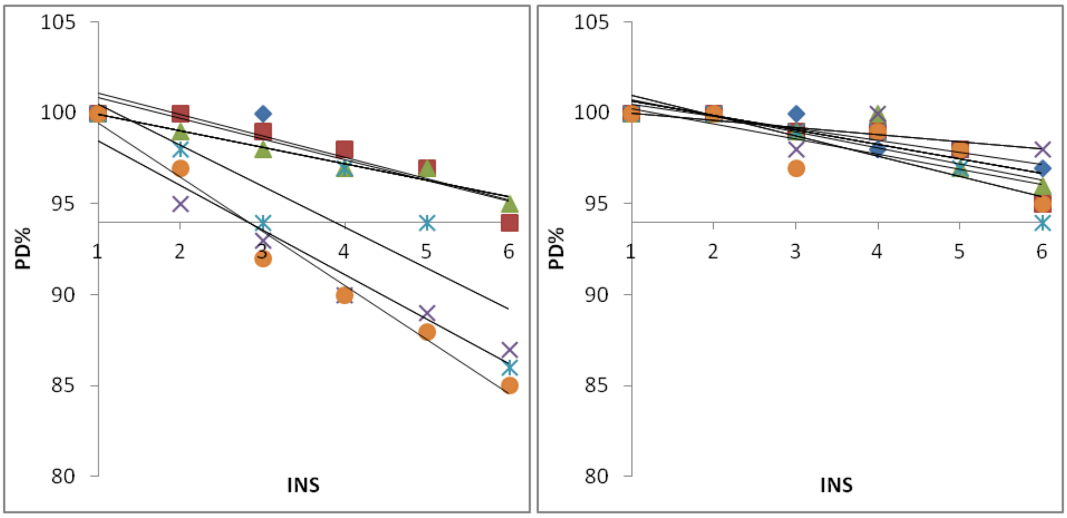
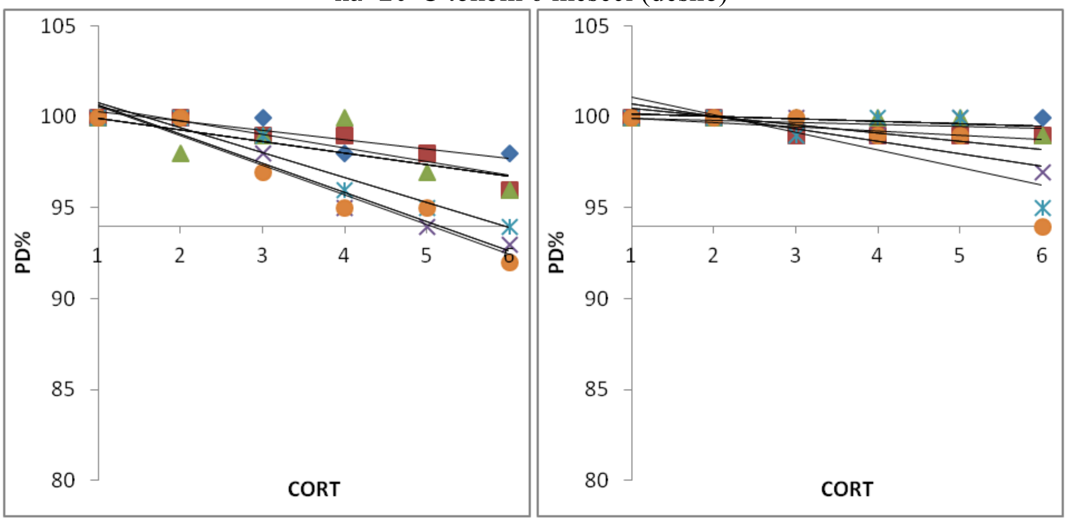
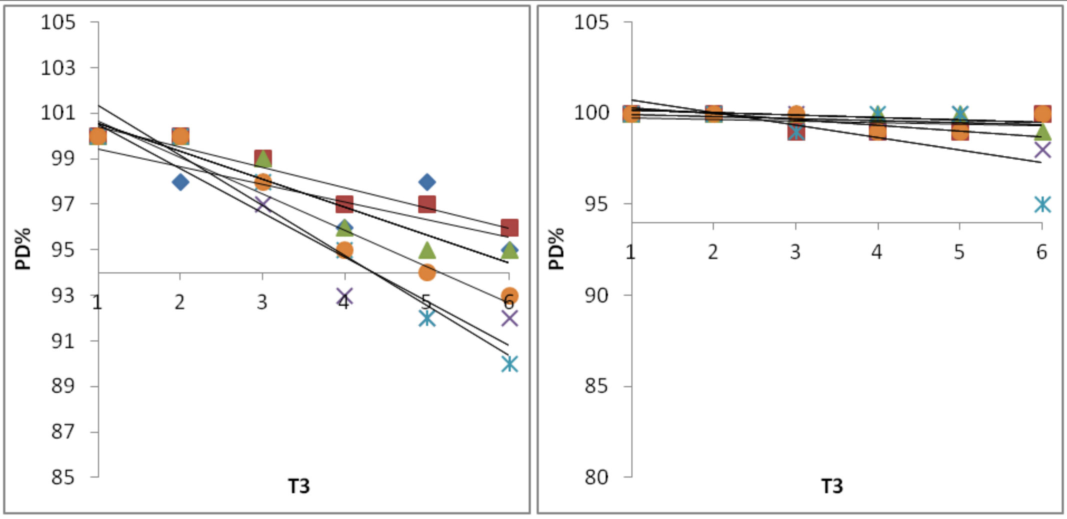
izgubio stabilnost ako se tokom veremena desi promena vrednosti parametara u procentu iznad ove maksimalno određene vrednosti. Najmanju stabilnost na +4°C pokazao je progesteron koji se može čuvati do 48h. Sledeći je bio insulin koji može biti stabilan do 3 dana u uzorku koji je opterećen preanalitičkim greškama, ali je bio stabilniji u uzorcima koji nisu bili opterećeni preanalitičkim grešakam. Kortizol, T3 i T4 imaju manju stabilnost pa se mogu čuvati do 4 ili 5 dana ako je uzorak opterećen preanalitičkim greškama. Kod zamrznutih uzoraka nije nađeno značajnije opadanje stabilnosti tokom eksperimentalnog perioda.



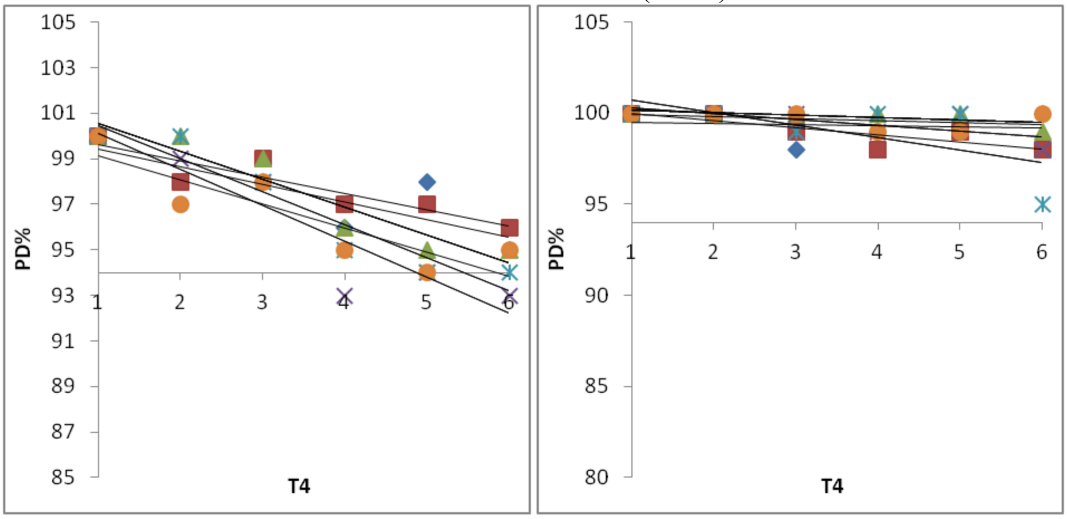
Grafikon 50 i 51: Stabilnost insulina (INS) na +4°C tokom 6 dana(levo) i na -20°C tokom 6 meseci (desno)



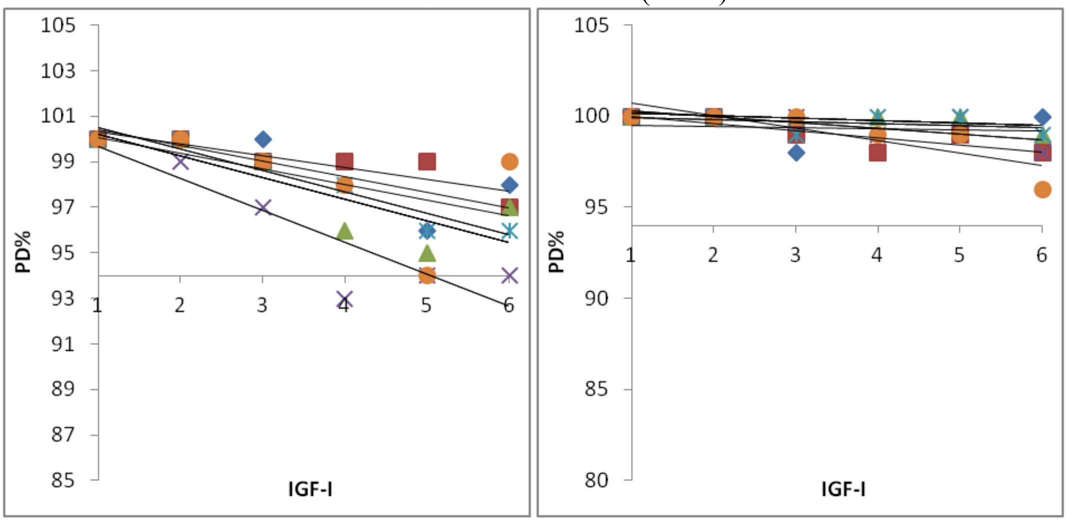
Grafikon 52 i 53: Stabilnost kortizola (CORT) na +4°C tokom 6 dana(levo) i na -20°C tokom 6 meseci (desno)



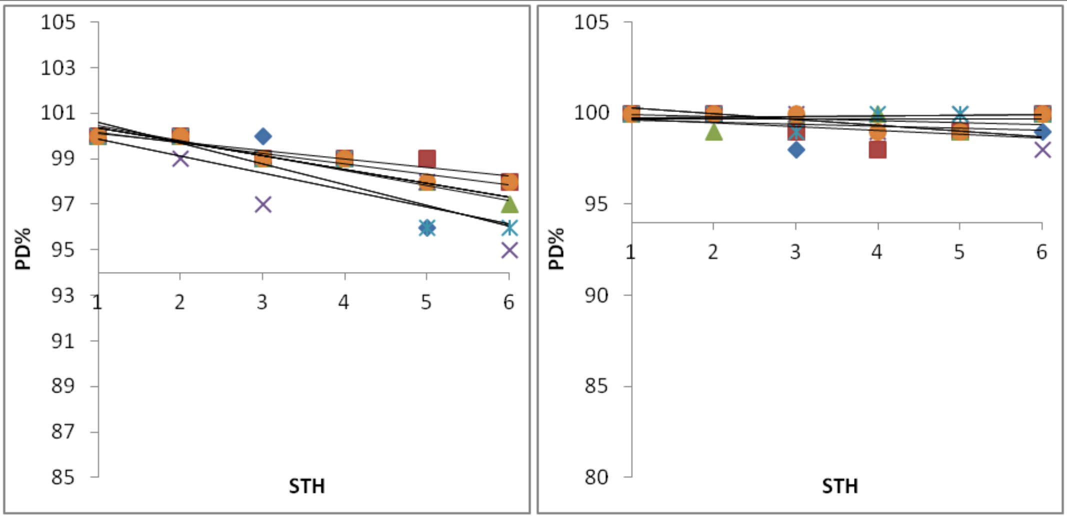
Grafikon 54 i 55: Stabilnost trijodotironina (T3) na +4°C tokom 6 dana(levo) i na -20°C tokom 6 meseci (desno)



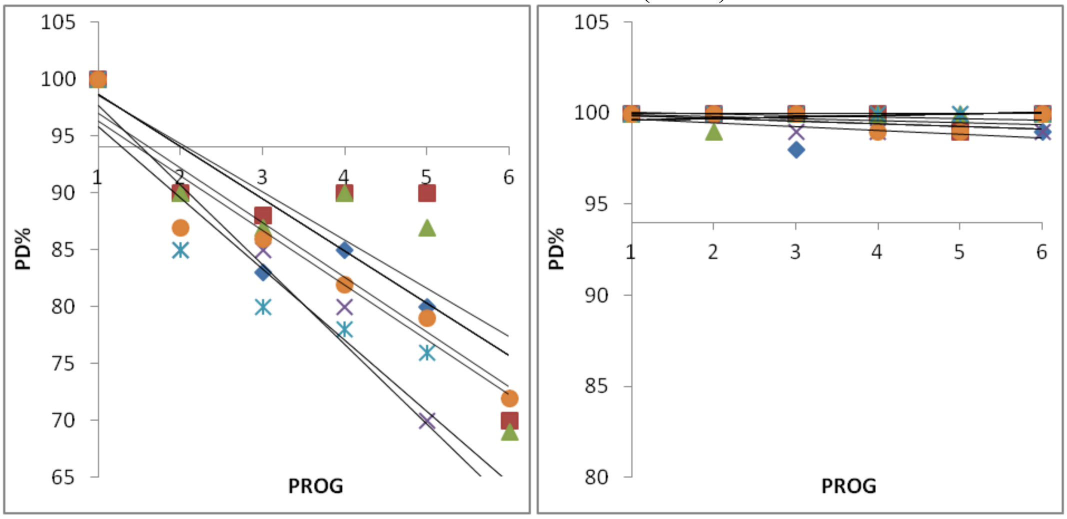
Grafikon 56 i 57: Stabilnost tiroksina (T4) na +4°C tokom 6 dana(levo) i na -20°C tokom 6 meseci (desno)



Grafikon 58 i 59: Stabilnost insulin sličnog faktora rasta I (IGF-I) na +4°C tokom 6 dana(levo) i na -20°C tokom 6 meseci (desno)



Grafikon 60 i 61: Stabilnost hormona rasta (STH) na +4°C tokom 6 dana(levo) i na -20°C tokom 6 meseci (desno)



Grafikon 62 i 63: Stabilnost progesterona (PROG) na +4°C tokom 6 dana(levo) i na -20°C tokom 6 meseci (desno)

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LITERATURA

1. *Cincović M. Metabolički stres krava. Monografija. Poljoprivredni fakultet Novi Sad, Departman za veterinarsku medicinu, 2016.*
2. *Belić B., Cincović M.R. Referentne vrednosti laboratorijskih parametara u krvi životinja. Monografija. Poljoprivredni fakultet Novi Sad, Departman za veterinarsku medicinu, 2020.*
3. *Đoković R.D.: Endokrini status mlečnih krava u peripartalnom periodu. Agronomski fakultet, Čačak, 2010.*
4. *Lippi G., Simundic A. Total quality in laboratory diagnostics. It's time to think outside the box. Biochemia Medica 2010, 20, 1, 5-8.*
5. *Lippi G., Bonelli P., Rossi Rossana, Bardi M., Aloe R., Galeffi A., Bonilauri E. Development of a preanalytical errors recording software. Biochemia Medica 2010, 20, 1, 90-95.*

ISBN 978-86-7520-611-8

6. *Braun J., Bourges-Abella N., Geffre A., Concordet D., Trumel C. The preanalytic phase in veterinary clinic pathology. Vet Clin Pathol 2015, 44, 1, 8-25.*
7. *Guder WG. History of the preanalytical phase: a personal view. Biochemia Medica 2014, 24, 1, 25-30.*
8. *Bowen R.A.R. Hortin G.L., Csako G., Otanez O.H., Ramaley A.T. Impact of blood collection devices on clinical chemistry assays. Clinical Biochemistry 2010, 43, 4-25.*
9. *Humann-Ziehank E., Ganter M. Pre-analytical factors affecting the results of laboratory blood analyses in farm animal veterinary diagnostics. Animal 2012, 6, 7.*
10. *Belić B., Cincović M.R., Došenović M., Stojanović D., Kovačević Z. The effects of different anticoagulants on biochemical parameters in blood of dairy cows. Veterinarski glasnik 2015, 69, 13-20.*
11. *Cray C., Rodriguez M., Zaias J., Altman N.H. Effects of storage temperature and time on clinical biochemical parameters from rat serum. Journal of the American Association for Laboratory Animal Science 2009, 48, 202–204.*
12. *Rasalkar K., Suma H.R. The impact of preanalytical variable like delay in sample transportation and post analytical variable like deep freezer temperature on combined Measurement Uncertainty. International Journal of Medical Science and Clinical Inventions 2017, 4, 3034-3036.*
13. *Bérubé E.R., Taillon M.P., Furtado M., Garofolo F. Impact of sample hemolysis on drug stability in regulated bioanalysis. Bioanalysis 2011, 3, 2097-2105.*
14. *Hooser S.B., McCarthy J.M., Wilson C.R., Harms J.L., Stevenson G., Everson R.J. Effects of storage conditions and hemolysis on vitamin E concentrations in porcine serum and liver. Journal of veterinary diagnostic investigation 2000, 12, 365-368.*
15. *Neuwinger N., Zum Büschenfelde D.M., Tauber R., Kappert K. Underfilling of vacuum blood collection tubes leads to increased lactate dehydrogenase activity in serum and heparin plasma samples. Clinical Chemistry and Laboratory Medicine (CCLM) 2020, 58, 213-221.*
16. *Mullins G.R., Bruns D.E. Air bubbles and hemolysis of blood samples during transport by pneumatic tube systems. Clinica Chimica Acta 2017, 473, 9-13.*
17. *Magee L.S. Preanalytical variables in the chemistry laboratory. In Lab notes. Becton, Dickinson and Company, Franklin Lakes, NJ, USA, 2005.*
18. *Kift R.L., Byrne C., Liversidge R., Babbington F., Knox C., Binns J., Barth J.H. The effect of storage conditions on sample stability in the routine clinical laboratory. Annals of clinical biochemistry 2015, 52, 675-679.*
19. *Rioja, R. G., Espartosa, D. M., Segovia, M., Ibarz, M., Llopis, M. A., Bauça, J. M., Marzana I., Barba N., Ventura M.,del Pino I.G., Puente J.J., Caballero A., Gómez C., Álvarez A.G., Alsina M.J., Álvarez V. Laboratory sample stability. Is it possible to define a consensus stability function? An example of five blood magnitudes. Clinical Chemistry and Laboratory Medicine (CCLM) 2018, 56, 1806-1818.*
20. *Clark S., Youngman L.D., Palmer A., Parish S., Peto R., Collins R. Stability of plasma analytes after delayed separation of whole blood: implications for epidemiological studies. International journal of epidemiology 2003, 32, 125-130.*
21. *Oddoze C., Lombard E., Portugal H. Stability study of 81 analytes in human whole blood, in serum and in plasma. Clinical biochemistry 2012, 45, 464-469.*
22. *Taylor E.C., Sethi B. Stability of 27 biochemistry analytes in storage at a range of temperatures after centrifugation. British journal of biomedical science 2011, 68, 147-157.*
23. *Tanner M., Kent N., Smith B., Fletcher S., Lewer M. Stability of common biochemical analytes in serum gel tubes subjected to various storage temperatures and times pre-centrifugation. Annals of clinical biochemistry 2008, 45, 375-379.*
24. *Donnelly J.G., Soldin S.J., Nealon D.A., Hicks J.M. Stability of twenty-five analytes in human serum at 22 degrees C, 4 degrees C, and -20 degrees C. Pediatr Pathol Lab Med 1995, 15, 869-874.*
25. *Jones D.G. Stability and storage characteristics of enzymes in cattle blood. Res Vet Sci. 1985a, 38, 3, 301-306.*
26. *Jones D.G. Stability and storage characteristics of enzymes in sheep blood. Res Vet Sci. 1985b, 38, 3, 307-311.*
27. *Ehsani A., Afshari A., Bahadori H., Mohri M., Seifi H.A. Serum constituents analyses in dairy cows: Effects of duration and temperature of the storage of clotted blood. Research in veterinary science 2008, 85, 473-475.*
28. *Moe M.O., Okstad W., Berland S., Framstad T. Effects of storage duration and temperature conditions on biochemical analytes in porcine clotted, uncentrifuged blood samples. J Dairy Vet Anim Res 2018, 7, 00181.*
29. *Stokol T., Nydam D.V. Effect of anticoagulant and storage conditions on bovine nonesterified fatty acid and β-hydroxybutyrate concentrations in blood. J Dairy Sci 2005, 88, 3139-3144.*
30. *Menéndez L.G., Fernández A.L., Enguix A., Ciriza C., Amador J. Effect of storage of plasma and serum on enzymatic determination of non-esterified fatty acids. Annals of clinical biochemistry 2001, 38, 252-255.*
31. *Morris J.D., Fernandez J.M., Chapa A.M., Gentry L.R., Thorn K.E., Weick T.M. Effects of sample handling, processing, storage and hemolysis on measurements of key energy metabolites in ovine blood. Small Rumin. Res 2002, 43,157–166.*

32. *Bach K.D., Neves R.C., Stokol T., McArt J.A.A. Effect of storage time and temperature on total calcium concentrations in bovine blood. J Dairy Sci 2020, 103, 922-928.*
33. *Flores C.F.Y., Pineda Á.D.L.M.H., Bonilla V.M.C., Sáenz-Flor K. Sample management: stability of plasma and serum on different storage conditions. EJIFCC 2020, 31 , 46.*
34. *Cincović M., Belić B., Radojičić B., Hristov S., Đoković R. Influence of lipolysis and ketogenesis to metabolic and hematological parameters in dairy cows during periparturient period. Acta veterinaria 2012, 62, 429-444.*
35. *Delić B., Belić B., Cincović M.R., Djokovic R., Lakić I. Metabolic adaptation in first week after calving and early prediction of ketosis type I and II in dairy cows. Large Animal Review 2020, 26, 2, 51-55.*
36. *Djoković R., Kurćubić V., Ilić Z., Cincović M., Lalović M., Jašović B., Bojkovski, J. Correlation between blood biochemical metabolites milk yield, dry matter intake and energy balance in dairy cows during early and mid lactation. Advances in Diabetes and Metabolism 2017, 5, 2, 26-30.*
37. *Young D.S., Bermes E.W. Specimen collection and processing: sources of biological variation. In: Burtis CA, Ashwood ER (eds). Tietz Textbook of Clinical Chemistry. Philadelphia: WB Saunders Company, pp. 42–72, 1999.*
38. *O'Connell T., Thornton L., O'Flanagan D. et al. Oral fluid collection by post for viral antibody testing. Int J Epidemiol 2001, 30, 298–301.*
39. *Perneger T.V., Etter J.F. Commentary: Extending the boundaries of data collection by mail. Int J Epidemiol 2001, 30, 301–302.*
40. *Ono T., Kitaguchi K., Takehara M., Shiiba M., Hayami K. Serumconstituents analyses: effect of duration and temperature of storage of clotted blood. Clin Chem 1981, 27, 35–38.*
41. *Hankinson S.E., London S.J., Chute C.G. et al. Effect of transport conditions on the stability of biochemical markers in blood. Clin Chem 1989, 35, 2313–2316.*
42. *Heins M., Heil W., Withold W. Storage of serum or whole blood samples? Effects of time and temperature on 22 serum analytes. Eur J Clin Chem Clin Biochem 1995, 33, 231–238.*
43. *Youngman L.D., Lyon V.E., Collins R. et al. Problems with mailed whole blood in large-scale epidemiological studies and methods of correction. The FASEB Journal 1993, 1, 377.*
44. *Sonntag O. Haemolysis as an interference factor in clinical chemistry. J Clin Chem Clin Biochem 1986, 24, 127–139.*
45. *Frank J.J., Bermes E.W., Bickel M.J. et al. Effect of in vitro hemolysis on chemical values for serum. Clin Chem 1978, 24, 1966–1970.*
46. *Gómez-Rioja R., Amaro M.S., Diaz-Garzón J., Bauçà J.M., Espartosa D.M., Fernández-Calle P. A protocol for testing the stability of biochemical analytes. Technical document. Clinical Chemistry and Laboratory Medicine (CCLM) 2019, 57, 12, 1829-1836.*
47. *Lippi, G.; Blanckaert, N.; Bonini, P.; Green, S.; Kitchen, S.; Palicka, V.; Vassault, A.J.; Plebani, M. Haemolysis: an overview of the leading cause of unsuitable specimens in clinical laboratories. Clin Chem Lab Med 2008, 46, 764–772.*
48. *Ward, G.; Simpson, A.; Boscato, L.; Hickman, P.E. The investigation of interferences in immunoassay. Clinical biochemistry 2017, 50, 18, 1306-1311.*
49. *Simundic, A. M.; Baird, G.; Cadamuro, J.; Costelloe, S. J.; Lippi, G. Managing hemolyzed samples in clinical laboratories. Critical reviews in clinical laboratory sciences 2020, 57(1), 1-21.*
50. *van Balveren J.A., Huijskens M.J., Gemen E.F., Péquériaux N.C., Kusters R. Effects of time and temperature on 48 routine chemistry, haematology and coagulation analytes in whole blood samples. Annals of clinical biochemistry 2017, 54, 448-462.*
51. *Kovačević, D., Cincović, M., Belić, B., Đoković, R., & Majkić, M. (2021). Blood serum stability limit and maximum storage time of bovine samples. Acta Scientiae Veterinariae, 49.*

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"DELTA CHECK" VREDNOST I NJENA KLINIČKA I LABORATORIJSKA UPOTREBA

"DELTA CHECK" VALUES AND ITS CLINIC AND LABORATORY USE

Vanja Kovačević[a*], Dražen Kovačević[a], Danijel Kovačević[a], Sandra Nikolić[b], Nemanja Obradović[c]

[a]Veterinarska ambulanta Kovačević, Teslić, R.Srpska, BiH
[b]Departman za veterinarsku medicinu, Poljoprivredni fakultet Novi Sad, Srbija
[c]Pasterov zavod, Novi Sad, Srbija
*Autor za korespodenciju: kovacevic.danijel8@gmail.com

S.N. https://orcid.org/0000-0001-7337-9937

SAŽETAK

Delta check vrednost je izmerena razlike između rezultata uzastopnih laboratorijskih testova iste jedinke. Kada je ta vrednost viša od očekivane, takav rezultat može da ukaže na problem u testiranju sa prethodnim ili trenutnim uzorkom i to treba blagovremeno istražiti, pre objavljivanja rezultata. Klasična formula za izračunavanje delta check je oduzimanje trenutne vrednosti parametra od prethodne vrednosti parametra za istu jedinku. Pored ovog razvijeni su i drugi pristupi izračunavanju delta check. Delta check procenat se izračunava tako što se razlika između trenutne vrednosti parametra i prethodne vrednosti pomnoži sa 100, a zatim se podeli sa trenutnom vrednošću parametra ili se izračuna količnik trenutne vrednosti parametra i prethodne vrednosti. U slučaju izračunavanja razlike između trenutne vrednosti i prethodne vrednosti parametra, ona mora da bude unutar granica koje se zovu delta check granice. U izračunavanju tih granica moramo uzeti u obzir: intraindividualne biološke varijacije za parametar koji se ispituje (koeficijent varijacije CV_i – odnos standardne devijacije prema srednjoj vrednosti); analitičke varijacije (SD – standardna devijacije, koja se lako može izračunati pomoću kontrolnih vrednosti, a predstavlja prosečno odstupanje od proseka); preanalitičke varijacije ($CV_{pre\text{-}analitic}$); poboljšanje ili pogoršanje zdravlja jedinke i greške u preanalitičkom, analitičkom ili postanalitičkom stadijumu rada.Kriterijumi u izboru analita za delta check imaju nekoliko ograničenja. Najčešće se koriste analiti koji imaju nizak koeficijent intra-individualnog variranja, što sprečava mogućnost lažne uzbune, ali smanjuje mogućnost za otkrivanje greške. Drugo ograničenje je interval u kom se delta check sprovodi, jer je on kratak, tako da su od koristi jedino testovi koji se često ponavljaju u kratkim vremenskim intervalima. Upotreba delta ček metoda je jednostavna metoda poređenja rezultata koja ukazuje na postojanje određenih značajnih promena u metaboličkom statusu jedinke.

Ključne reči: krave, metabolički profil, delta check, kontrola kvaliteta.

ABSTRACT

The delta check value is the measured difference between the results of consecutive laboratory tests of the same individual. When this value is higher than expected, such a result may indicate a problem in testing with the previous or current sample and should be investigated in a timely manner, before the results are published. The classic formula for calculating delta check is subtracting the current parameter value from the previous parameter value for the same individual. In addition to this, other approaches to calculating the delta check have been developed. The delta check percentage is calculated by multiplying the difference between the current value of the parameter and the previous value by 100 and then dividing by the current value of the parameter or calculating the quotient of the current value of the parameter and the previous value. In the case of calculating the difference between the current value and the previous value of the parameter, it must be within limits called delta check limits. In calculating these limits, we must take into account: intraindividual biological variations for the parameter being examined (coefficient of variation CVi – the ratio of standard deviation to the mean value); analytical variation (SD – standard deviation, which can be easily calculated using control values, and represents the average deviation from the average); pre-analytic variations (CVpre-analytic); improvement or deterioration of the individual's health and errors in the pre-analytical, analytical or post-analytical stages of work. The criteria for choosing an analyte for delta check have several limitations. Analytes that have a low coefficient of intra-individual variation are most often used, which prevents the possibility of a false alarm, but reduces the possibility of detecting an error. Another limitation is the interval in which the delta check is performed, because it is short, so only tests that are repeated frequently in short time intervals are useful. The use of the delta check method is a simple method of comparing results that indicates the existence of certain significant changes in the metabolic status of an individual.

Key words: cow, metabolic profile, delta check, quality control.

DEFINICIJA I ZNAČAJ DELTA CHECK METODE

Delta check je merenje razlike između rezultata uzastopnih laboratorijskih testova iste jedinke. Kada je ta vrednost viša od očekivane, takav rezultat može da ukaže na problem u testiranju sa prethodnim ili trenutnim uzorkom i to treba blagovremeno istražiti, pre objavljivanja rezultata. Ovaj metod je najbolji koji je trenutno dostupan za otkrivanje pogrešne identifikacije uzorka, a može nastati u svim fazama rada i odnosi se na upoređivanje rezultata biohemijskih i hematoloških analiza dva ili više uzoraka krvi, koji potiču od iste jedinke, a uzeti su u određenom vremenskom razmaku (1). *Delta check* se može izraziti kao apsolutna vrednost ili procenat razlike između dva uzastopna rezultata. Apsolutna vrednost se izračunava oduzimanjem manje vrednosti rezultata od veće, dok se delta procenat računa oduzimanjem manjeg rezultata od većeg i zatim se razlika podeli sa manjom vrednošću. Razlika između dva seta rezultata upoređuje se sa granicom, koja je specifična za merenje (1). *Delta check* je u širokoj upotrebi u kliničkim laboratorijama kao alat za procenu kvaliteta na osnovu istih jedinki, kako bi se pronašle greške u vezi sa skupljanjem uzoraka, u toku analize ili u izveštavanju. Omogućava sigurnu mrežu za identifikaciju grešaka koje bi u suprotnom prošle neopaženo i veoma je važan deo auto-verifikacijskih procedura za unapređivanje efikasnosti rada laboratorije.

Procedure kontrole kvaliteta su tako dizajnirane da otkrivaju greške koje se dešavaju u toku analize uzorka, ali su takve procedure neefikasne u otkrivanju pogrešno identifikovanih uzoraka ili grešaka koje se mogu dogoditi u periodu između analize i izveštavanja o rezultatima testova. *Delta check* koncept je nastao pre upotrebe traka za ruke pacijenata sa bar-kodovima, označavanja epruveta bar-kodovima i moderne, rigorozne identifikacije pacijenata, koja je sada široko rasprostranjena. Usled nedostatka ovakvih procedura bilo je mnogo više grešaka nego danas, učestalo je bilo pogrešno označavanje epruveta za primarno sakupljanje uzoraka, a alikvoti su morali biti znatno veći (2).

Verovatno je prva preporuka za upotrebu *delta check*-a bila 1967. godine kao deo predloženog laboratorijskog kompjuterskog algoritma za otkrivanje neuobičajenih zapažanja, u cilju prepoznavanja problema u kvalitetu, koji se dešavaju pre i posle testiranja (2,3). Među ovim zapažanjima je bilo rezultata testova za koje se smatra da nisu kompatibilni sa životom jedinke, zatim promena u rezultatima testova, koje prelaze određene granice (koncept *delta check*) i prepoznati su šabloni zasnovani na poznatim vezama među različitim testovima na istom uzorku (2).

Ovaj koncept je 1974. godine uvedena (4) kao tehniku kontrole kvaliteta kako bi se pronašli pogrešno identifikovani uzorci. Retrospektivno su opisali ispitivanja serije uzastopnih laboratorijskih rezultata kompletne krvne slike i razlike za svakom pojedinačnog pacijenta, svaki put kad je rađena nova analiza, sa ciljem da se otkriju odstupanja pre nego što rezultati napuste laboratoriju. Oni su ručno proveravali trenutne i prethodne rezultate od određenih pacijenata kako bi pronašli neverovatne promene u rezultatima laboratorijskih procedura. Greške koje su pronašli su uglavnom bile administrativne i greške u identifikaciji uzoraka. Većina *delta check* upozorenja je bila stvarna promena u stanju pacijenta (98%), a procedura je

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uvela nove troškove za laboratoriju (2). 1975. godine *Ladenson* je opisao prvu upotrebu kompjutera za upoređivanje trenutnih i prethodnih uzoraka u realnom vremenu kao prikazane rezultate. Ovaj opšti pristup u pronalaženju značajnih *delta check*-ova se nije mnogo promenio u narednih 40 godina.[54] On je koristeći kompjuter i odabrane kliničke biohemijske i serološke testove, razmotrio, ali nije usvojio, pragove koji se zasnivaju na biološkim varijacijama, što je naknadno uradio *Young et al* (2,5).[52,55] Istovremeno *Whitehurst et al.* opisuju sistem koji uključuje rutinske biohemijske testove, ali ispituje promenu u izračunatom anjonskom jazu, što je prvi multivarijantni pristup *delta check*-u (2,6). Od tada su svi pristupi određivanju *delta check* pragova (granica) postavljani empirijski. *Wheeler and Sheuner* su koristili arhivirane laboratorijske podatke da odrede mogućnost promene šest učestalo merenih biohemijskih testova i dva izračunata parametra, anjonski jaz i odnos uree i kreatinina (2,7). Ovaj pristup je znatno komplikovaniji u odnosu na prethodne, jer su se odreŝivali i koristili *delta check* pragovi zasnovani na kategorijama rezultata testova za svaki trenutni rezultat, koristeći dva različita vremenska intervala među uzorcima i sedam različitih *delta check pragova* zasnovanih na mogućnosti greške za koje su pripisane različite radnje. U svim studijama tokom prethodnih decenija je pokazano da svi pristupi imaju slične mogućnosti u učestalosti pojave *delta check* upozorenja i otkrivanja greške. Kao izazov u ovim studijama je održavanje ravnoteže u otkrivanju grešaka i rada koji je potreban za procenu i isključenje greške, u slučajevima promena većine rezultata kod iste jedinke objašnjavane su patofiziološkim faktorima ili kliničkim intervencijama.

PRAKTIČNA UPOTREBA DELTA CHECK METODE

Metodom *delta check* mogu se identifikovati greške koje se ne mogu ustanoviti standardnim metodama kontrole kvaliteta. Prekomerno i nepravilno korišćenje *delta check* metoda može dovesti do odlaganja izdavanja rezultata i povećanja opterećenja u laboratoriji zbog potrebe za dodatnim manuelnim potvrđivanjem rezultata. Korišćenjem odgovarajuće metode *delta check*-a, dodatno se smanjuje opterećenje i povećava se brzina u pronalaženju greške (5). Većina laboratorija koristi neku od 4 standardne metode *delta check*-a, a to su delta razlika, delta procent promene, stopa razlike i stopa procenta promene (6-10). U odlučivanju koja vrsta *delta check*-a će se koristiti glavni faktori su vremenska zavisnost i obim varijacija. U slučaju da je obim varijacija prilično veliki, koristi se delta razlika ili stopa procenta promene, jer u koliko bi se koristila stopa razlike, došlo bi do velikog opterećenja zbog širine obima. U slučajevima kada obim varijacije nije veliki, koristićemo delta razliku ili stopu razlike. U zavisnosti od vrste analita koji se ispituje, vremenska zavisnost je bitna, jer se određeni parametri menjaju na dnevnom nivou, a određeni čak na mesečnom. Najveći izazov u odabiru metode je nepostojanje tačno određenog kriterijuma za odabir *delta check* metode koja odgovara predmetu testiranja. *Delta check* granica definiše dozvoljenu razliku između dva uzastopna rezultata za isti parametar. Vremenska razlika između dva merenja je fleksibilna, u većini laboratorija ta razlika je od 24h do 48h. U osnovi, bitno je izabrati hematološke i biohemijske parametre koji imaju najnižu biološku varijaciju.

DELTA CHECK METODA KOD KRAVA

Na variranje biohemijskih i hematoloških parametara u krvi krava utiče veliki broj faktora (1,11,12,13,14,15). Svi faktori se po vremenu nastanka dele na preanalitičke, analitičke i postanalitičke. Preanalitički faktori su hemoliza, temperatura, antikoagulansi, laktacija, stres, ishrana, određena patološka stanja i upotreba lekova. Najčešće greške u ovom stadijumu ispitivanja su neodgovarajući uzorak, tj. neodgovarajući odnos uzorka i antikoagulansa, pogrešan antikoagulans, neodgovarajući metod čuvanja uzorka, pogrešna prirpema pacijenta, na primer pogrešna ishrana pred vađenje krvi, greške u identifikaciji pacijenta (16). Na primer, u hemoliziranim uzorcima opada vrednost hematokrita, broj eritrocita i MCV, a raste MCH, MCHC i broj trombocita. Od biohemijskih parametara najveće promene usled hemolize pokazuju AST, ukupni bilirubin, trigliceridi i NEFA, dok glukoza, BHB i ukupni proteini pokazuju malo odstupanje. U hemoliziranim uzorcima postoji tendencija rasta vrednosti albumina, AST, ALT i niže vrednosti kreatinin kinaze (CK), kalcijuma i magnezijuma. MCHC je pokazatelj hemolize i za promenu od jedne jedinice najviše se menja vrednost glukoze, ALP, AST, ALT, CK, Mg, Ca i bilirubina. Postojanje hemolize može da oteža analizu metaboličkog profila krava, ali je njen uticaj minimalan ukoliko se uzorak transportuje na stabilnoj temperaturi.

Kod korišćenja antikoagulanasa, u zavisnosti od vrste koja se koristi, dolazi do promena u biohemijskim parametrima u odnosu na vrednosti biohemijskih parametara u serumu. Kod uzoraka, kod kojih je korišćen heparin nađena je viša vrednost albumina (+4,1%) i ukupnih proteina (1,4%), a niža vrednost alkalne fosfataze (AP) u odnosu na serum (-33%). U uzorcima kod kojih je korišćen etilendiamin tetrasirćetna kiselina (EDTA), dolazi do pada ukupnih proteina (-5,8%), Ca (-49,6%), P (-17,7%), AP (-32%) i viša vrednost AST (10,6%) u odnosu na serum. U uzorcima gde su primenjeni citrat i fluorid, nađena je niža vrednost ukupnih proteina, albumina, glukoze (samo citrat), Ca, P, BHB, NEFA, uree (samo citrat), holesterola, AP i GGT (samo fluorid) i vrednost bilirubina u slučaju citrata opada, a raste u slučaju fluorida. Na osnovu ovih podataka, za biohemijsku analizu, najpouzdanije je koristiti heparin kao antikoagulans, jer su sa heparinom najmanja odstupanja u vrednosti parametara u odnosu na serum.

Kod krava je veoma bitan period u kom je uzeta krv, da li je to u periodu zasušenja, rane laktacije ili sredine laktacije. Zbog velikih metaboličkih promena, naročito u peripartalnom periodu, smanjenog unosa hrane, negativnog energetskog bilansa, kao i mobilizacije lipida i izmenjene funkcije jetre i hepatocita, postoje velike razlike u vrednostima metabolita. Kod uzorkovanja neposredno posle obroka u serumu opada koncentracija NEFA i BHB, dok su ostali metabolički parametri stabilni. Metabolički i hematološki profil krava zavisi od stepena kataboličke opterećenosti krava tokom rane laktacije. Klasifikacija krava prema kriterijumu NEFA pokazuje da krave opterećene metaboličkim stresom (krave sa višim vrednostima NEFA u prvoj nedelji po teljenju) imaju višu koncentraciju hormona rasta, BHB, AST, ALT, GGT, AP, bilirubina, a nižu koncentraciju ukupnih proteina, uree, holesterola, triglicerida, Ca, Mg, P.

DELTA CHECK I LABORATORIJSKA MEDICINA

Analitički faktori koji utiču na variranje parametara u krvi su najmanji izvor varijacije laboratorijskih parametara, jer se analitička faza ispitivanja odvija u laboratoriji i najmanja je verovatnoća greške. U kliničkim laboratorijama udeo grešaka u analitičkoj fazi obrade uzoraka je 4-13% (17). Najčešće greške u ovom stadijumu rada su istekli rokovi reagenasa, isteklo vreme kontrole ili kalibracije, kvar u sistemu uzorkovanja, kvar u delu za aspiraciju reagensa, promete u fotometrijskoj jedinici, protočnoj ćeliji ili mernoj jedinici analizatora i druge greške analizatora (16). Najbitnije je u toku laboratorijske analize da se priprema i postupa sa uzorkom na strogo propisan način. Greške koje se javljaju u ovoj fazi rada su u vezi sa greškom opreme, izgubljenim uzorkom ili analitičkom greškom. S tim u vezi, aparati treba da budu redovno proveravani sa aspekta unutrašnje kontrole kvaliteta, a potrebna je i redovna kalibracija. Varijacija u vrednostima je neizbežna i zavisi od vrste analitičkog testa. U specifikaciji aparata odnosno analitičkih testova obavezno postoji inter i intraesejski koeficijent varijacije. Sve varijacije iz svih izvora se sabiraju i utiču na ukupnu varijaciju koja se procenjuje *delta check* metodom (1).

Postanalitički faktori mogu uticati na promene u parametrima krvi u slučaju određenih grešaka. U kliničkim laboratorijama udeo grešaka u postanalitičkoj fazi je 19-47% i to su greške u slučaju izgubljenih rezultata, greške u prepisivanju, vreme izdavanja rezultata i greške u tumačenju nalaza (17). Iz ovih razloga bitno je da se po prijemu rezultata iz laboratorije sprovedu sledeće procedure: odobrenje od strane veterinara odnosno nadređenog laboranta, da se obezbedi tehnička kontrola svih relevantnih naučnih i tehničkih podataka, postojanje dokumentacije o proveri podataka, neželjeni podaci, greške i druge nepravilnosti od strane primenjenih programa i svaki korak u postupku se dokumentuje, a greške se eliminišu, sve implikacije o nezadovoljavajućim rezultatima testova se uzimaju u obzir, a greške se ispravljaju, dok se metode ispravke dokumentuju i čuvaju kao izvor informacija.

U laboratorijama, kako u humanoj tako i u veterinarskoj medicini, koriste se automatizovani analizatori. Oni su veoma prefinjeni instrumenti, koji mogu uraditi veliki broj laboratorijskih testova u kratkom vremenskom periodu. Spoj analitičke hemije, informatike i robotike je doveo do stvaranja ovakve tehnologije i obim posla osoblja u laboratorijama je značajno smanjen. Laboratorijsko osoblje sada rukuje opremom, vrši interne i eksterne kontrole, kalibraciju instrumenata i obradu podataka

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iz dobijenih rezultata. Kako bi se izbegle greške u rezultatima i radu potrebno je vršiti kontrolu kvaliteta. Moderna kontrola kvaliteta koristi minimalni broj uzoraka kako bi se uradila provera i taj proces se naziva statistička kontrola kvaliteta (SQC). Ona se definiše kao proces koji se fokusira na otkrivanje bilo kakvih odstupanja od dobro definisanih standarda (16). U kliničkim laboratorijama, zbog velikih bioloških varijacija i drugih uticaja koji mogu promeniti rezultate, SQC se ne može raditi za svaki rezultat posebno, već za određeni instrument ili analitičku metodu. Analitičke greške možemo podeliti na slučajne greške, kod kojih nemamo definisan uzrok greške ili imamo dobro definisan uzrok, i na sistemske greške, koje su komponenta greške, koja u toku niza analiza iste merne veličine, ostaje konstanta ili varira na predvidiv način. *Delta check* je metoda kojom detektujemo slučajne greške, koristeći prethodnu vrednost parametra za određenu jedinku.

Klasična formula za izračunavanje *delta check* je oduzimanje trenutne vrednosti parametra od prethodne vrednosti parametra za istu jedinku. Pored ovog razvijeni su i drugi pristupi izračunavanju *delta check*. *Delta check* procenat se izračunava tako što se razlika između trenutne vrednosti parametra i prethodne vrednosti pomnoži sa 100, a zatim se podeli sa trenutnom vrednošću parametra ili se izračuna količnik trenutne vrednosti parametra i prethodne vrednosti. U slučaju izračunavanja razlike između trenutne vrednosti i prethodne vrednosti parametra, ona mora da bude unutar granica koje se zovu *delta check* granice. U izračunavanju tih granica moramo uzeti u obzir:

- intraindividualne biološke varijacije za parametar koji se ispituje (koeficijent varijacije CV_i – odnos standardne devijacije prema srednjoj vrednosti)
- analitičke varijacije (SD – standardna devijacije, koja se lako može izračunati pomoću kontrolnih vrednosti, a predstavlja prosečno odstupanje od proseka)
- preanalitičke varijacije ($CV_{pre-analitic}$)
- poboljšanje ili pogoršanje zdravlja jedinke
- greške u preanalitičkom, analitičkom ili postanalitičkom stadijumu rada (16).

Peripartalni period kod krava predstavlja najznačajniji period za ispitivanje metaboličkog, zdravstvenog i produktivnog statusa krava. U ovom periodu se zbog negativnog energetskog bilansa i započinjanja laktacije dešavaju brojne metaboličke promene (18-19). Dinamičke metaboličke promene kod mlečnih krava u peripartalnom periodu izučavaju se frekventnim uzimanjem krvi, na nedeljnom ili dnevnom nivou, a iz tih uzoraka se određuju različiti krvni parametri. Poređenjem dobijenih rezultata tokom vremena uzimanja dolazi se do zaključka da metabolizam krava pokazuje određenu "metaboličku plastičnost" u prilagođavanju i da se krave međusobno razlikuju po stepenu odgovora na negativni energetski bilans (20). Klasifikacija krava na osnovu intenziteta lipolize, ketogeneze, promene telesne kondicije, odnosa kataboličkih i anaboličkih indikatora i drugo, daje značajne razlike u odstupanju parametara krvne slike i metaboličkog profila u prvim nedeljama laktacije (21-25). Postojanje individualnih razlika u odgovoru na metabolički stres u ranoj laktaciji otvara mogućnost da se pored referentnih intervala zasnovanih na populaciji mogu koristiti i referentne vrednosti zasnovane na jedinci, uz longitudnalno praćenje jedne životinje kroz određeno vreme da bi se ispitala plastičnost njenog metabolizma.

Kod određivanja intraindividualnog variranja rezultata cilj je da se odredi referentna vrednost metaboličkih parametara za svaku jedinku posebno (26). Ukoliko je intraindividualno variranje parametara manje od variranja u populaciji, u procesu procene metaboličkog statusa u obzir bi trebale da se uzmu intraindividualne vrednosti. Suprotno, ako su intraindividualne varijacije slične onima u populaciji, u obzir se prilikom interpretacije mogu uzeti interindividualne referentne vrednosti populacije. U tu svrhu se izračunava *index of individuality* i *subject-based reference values* kao što je *the reference change value* (RCV) (27).

RCV je jedan od pristupa u izračunavanju *delta check* granica i zove se i kritična promena (*critical change*) (2,28) i za njegovo izračunavanje se najčešće koristi prethodni tj.prvi rezultata određenog testa kao osnova za određivanje promene u uzastopnim uzorcima. Tada se RCV upoređuje sa uspostavljenim statističkim granicama. Izračunavanje RCV se može uraditi na najmanje pet načina, ali sve u zavisnosti od toga da li je promena koja je nastala dovela do povećanja ili smanjenja rezultata, da li je prvi rezultat koji se koristi kao osnova u homeostatskim referentnim vrednostima, od prvog i drugog rezultata ili od drugih procena i da li je distribucija rezultata za određeni analit normalna ili je njegov logaritam normalan u odnosu na nastalu promenu (2,29). U određivanju RCV

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razmatra se analitička nepreciznost za određeni analit i intra-individualna varijabilnost kao sastavni deo bioloških varijacija. Određivanje RCV na prvom mestu zavisi od izbora verovatnosti, koja je uglavnom od 95% do 99% rezultata. Inter-individualne varijacije definišu se kao varijacije homeostatskih referentnih vrednosti u grupi jedinki u populaciji (2).

Analiti za koje je ova vrednost niska, obično ispod 0,6, su pogodniji za otkrivanje grešaka u slučajevima mešanja uzoraka (30,31). Iz tog razloga su ALT, MCV, MCH, protrombinsko vreme i kreatinin, čiji je *index of individuality* ispod 0,4, bolji od ostalih analita gde je preko 0,6. Razlike u nepreciznosti testova u različitim laboratorijama zajedno sa različitim procenama bioloških varijacija kroz istraživanja mogu pokazati značajno različite proračune *index of individuality* (32). Zbog svega navedenog ovaj pristup treba koristiti pažljivo i procenjivati analitičke razlike u performansama određene laboratorije, pre generalizovanja pravila *delta check*-a i različitih kriterijuma u laboratorijama. Pored svega, veoma je važan izbor analita za *delta check* kao što su granice za promene gde se vrednost parametra poveća (promena na gore) u odnosu na promene gde se vrednost parametra smanji (promena na dole), kako bi se usavršilo otkrivanje grešaka i smanjio broj lažnih uzbuna.

Osnovni razlozi za korišćenje *delta check* analize su identifikacija uzorka i problemi koji se javljaju pri sakupljanju uzoraka, a koji utiču na preanalitički, analitički i postanalitički kvalitet, a koji nisu otkriveni kontrolom kvaliteta, i pomoć kliničarima u otkrivanju bolesti i značajnih promena u stanju pacijenta. *Delta check* se koristi i za otkrivanje administrativnih grešaka, mešanja uzoraka, kontaminacije uzoraka, pogrešno rukovanje uzorcima, korišćenje pogrešnog antikoagulanta, prisustvo hemolize i druge interferencije, zatim problemi u pripremi uzoraka, instrumenata i reagenasa. U praksi, prava biološka promena kod pacijenta je glavni opis *delta check* vrednosti koje prekoračuju granice, naročito kad se granice zasnivaju na RCV. Kada se koristi u situacijama otkrivanja pogrešnog označavanja uzorka, efikasnost koncepta *delta check* se zasniva na dva preduslova. Prvi je da je razlika u rezultatima uzastopnih testova na uzorcima istog pacijenta manja nego razlika u rezultatima kod različitih pacijenata i drugi preduslov je da velika kratkotrajna promena nije uobičajena za jedinku. Ovo su veliki izazovi u praksi. Nagle promene, koje su kratkotrajne, a prelaze tipičnu kontrolnu granicu *delta check* su uobičajene za akutne bolesnike i pacijente koji su podvrgnuti određenim medicinskim tretmanima. Uzorci koji su pomešani u preanalitičkoj fazi, uglavnom prolaze neopaženo, ako razlike u rezultatima među pacijentima nisu velike. Ipak klinički značajna promena koja dovodi do pojave *delta check* uzbune pruža mogućnost za istraživanje i otkrivanje pogrešno identifikovanih uzoraka, koji ukoliko se ostave neotkriveni mogu odvesti u neodogovarajući tretman i upravljanje rezultatima. Savršena strategija otkriva sve pogrešne rezultate testova pre njihovog izdavanja. Usvojene strategije se zasnivaju na potrebama određenih laboratorija, ukupne stope njihovih grešaka i mogućnosti osoblja da ponovo pregleda *delta check* rezultate (2,33).

Pored svega pogrešno obeležavanje uzoraka ostaje jedna od najčešćih preanalitičkih grešaka otkrivenih *delta check*-om, a njihov uzrok je pogrešna identifikacija paijenta, što utiče na 72% negativnih ishoda u medicinskim laboratorijama (34). Kroz različita ispitivanja je pokazano da je 4,8% *delta check* uzbuna povezano sa problemima u toku testiranja, uglavnom zbog kontaminacije uzoraka i interferencija, a samo 0,3% *delta check* uzbuna su u vezi sa pogrešnom identifikacijom uzorka. U skoro 95% slučajeva u kojima je došlo do *delta check* uzbune nije došlo do promena rezultatat testa i nije utvrđen problem. Najveće pitanje u korišćenju *delta check* je da li je broj otkrivenih grešaka vredan ulaganja u rad i druge troškove ispitivanja *delta check* uzbuna.

Kriterijumi u izboru analita za *delta check* imaju nekoliko ograničenja (35). Najčešće se koriste analiti koji imaju nizak koeficijent intra-individualnog variranja, što sprečava mogućnost lažne uzbune, ali smanjuje mogućnost za otkrivanje greške. Drugo ograničenje je interval u kom se *delta check* sprovodi, jer je on kratak, tako da su od koristi jedino testovi koji se često ponavljaju u kratkim vremenskim intervalima, što upotrebu ograničava na bolničke pacijente i klinike u kojima su testiranja česta. Najčešće korišćeni analiti u *delta check* šemi su MCV, hemoglobin, broj trombocita, natrijum, kalcijum, kalijum, kreatinin, urea, albumini i proteini. *Delta check* uzbuna za natrijum, kalijum, kalcijum, magnezijum, MCV, MCH, MCHC, hemaglobin, hematokrit i broj trombocita su bili u vezi sa višom stopom otkrivenih grešaka (36). Najmanju efikasnost su imali ukupni proteini, bilirubin direktni i ukupni, mokraćna kiselina, AST, AP, glukoza, LDH, GGT i holesterol.

Optimalan sistem je onaj gde je broj lažnih *delta check* uzbuna minimalan sa prihvatljivom stopom otkrivenih grešaka. Pored svih pristupa i izračunavanja, i dalje postoje velike nedoumice u vezi sa korišćenjem *delta check-a* za otkrivanje grešaka. Zahtevi koji se moraju ispuniti su pažljiv i uravotežen pristup u odabiru, implementaciji, nadzoru, pregledu i ažuriranju, u skladu sa saznanjima, na putu ka otkrivanju grešaka ili bolesti, ali na način koji minimalizuje negativne uticaje lažno pozitivnih uzbuna. Optimalan pristup *delta check* je prilagođen za pojedinačne laboratorije, gde se uzroci i rezultati *delta check* uzbuna blagovremeno prate i kontrolišu i deo su procesa unapređenja. Metabolička istraživanja kod preživara i veliki značaj laboratorijske medicine u donošenju svakodnevnih odluka, posebno kod visokomlečnih krava (37) zahteva usvajanje novih laboratorijskih indikatora zdravlja i homeostaze životinja, a jedan od njih je delta check metod. Delta check metod procene rezultata u laboratorijama definisan je i međunarodnim laboratorijskim standardima (38), pa smernice treba uvesti u svakodnevnu veterinarsku laboratorijsku i dijagnostičku praksu.

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REFERENCE

1. *Cincović M.R., Belić B, Starič J., Ježek J. Poboljšani postupak identifikacije porekla uzoraka krvi krava pomoću hematoloških i biohemijskih parametara čije je variranje odeđeno „Delta check“ metodama. Departman za veterinarsku medicinu, Poljoprivredni fakultet, Novi Sad, 2017.*
2. *Edward W.R., Sedef Y. Delta check in the clinical laboratory. Critical Reviews in Clinical Laboratory Sciences, 2018.*
3. *Lindberg D.A. Collection, evaluation and transmission of hospital laboratory data. Methods of Information in Medicine 1967, 6, 97-107.*
4. *Wayne P.A. Use of Delta Checks in The Medical laboratory Clinical and Laboratory standard Institute guideline,Vol.1, EPP 33, Clinical and Laboratory Standard Institute, 2016.*
5. *Young D.S., Harris E.K., Cotlove E. Biological and analytic components of variations in long-term studies of serum constituents in normal subjects: IV. Results of a study designed to eliminated long-term analytic deviations. Clinical Chemistry 1971, 17, 403-410.*
6. *Whitehurst P., Di Silvio T.V., Boyadjian G. Evaluation of discrepancies in patients results - an aspect of computer-assisted quality control. Clinical Chemistry 1975, 21, 87-92.*
7. *Wheeler L.A., Sheiner L.B. Delta check tables for the Technicon SMA 6 continuous-flow analyzer. Clinical Chemistry 1977, 23, 216-219.*
8. *Sang H.P., So-Young K., Woochang L., Sail C., Won-Ki M. New decision criteria for selecting delta check methods based on the ratio of delta diference to the width of the reference range can be generally applicable for each clinical chemistry test item. Annals of Laboratory Medicine 2012, 32, 5, 345-354.*
9. *Kim J.W., Kim J.Q., Kim S.I. Differential application of rate and delta check on selected clinical chemistry tests. Journal of Korean Medical Science 1990, 5, 189–195.*
10. *Lacher D.A., Connelly D.P. Rate and delta checks compared for selected chemistry tests. Clinical Chemistry 1988, 34, 1966-1970.*
11. *Min W.K., Lee J.O. The development of online delta check system using the workstation. Journal of Clinical Pathology Quality Control 1996, 18, 133-142.*
12. *Leniček Krleža J.: Laboratorijska medicina: predanalitička pogreška, interferencije i interpretacija laboratorijskih nalaza. Klinika za dječije bolesti Zagreb, Osjek, 2014.*
13. *Belić B., Cincović M.R., Došenović M., Stojanović D., Kovačević Z. The effects of different anticoagulants on biochemical parameters in blood of dairy cows. Veterinarski glasnik 2015, 69(1-2), 13-20.*
14. *Kovačević D., Cincović M., Belić B., Đoković R., Majkić M. Blood serum stability limit and maximum storage time of bovine samples. Acta Scientiae Veterinariae 2021, 49.*
15. *Cincović M.R., Starič J., Belić B., Ježek J., Lakić, I. Influence of blood sample hemolysis on hematological and biochemical parameters in cows during early lactation. Contemporary Agriculture 2016, 65(3-4), 39-43.*

ISBN 978-86-7520-611-8

16. *Sheiner L.B., Wheeler L.A., Moore J.K. The performance of delta check methods. Clinical Chemistry 1979, 25: 2034-2037.*
17. *Karkalousos P., Evangelopoulos A. Quality Control in Clinical Laboratories, Applications and Experiences of Quality Control, Prof. Ognyan Ivanov (Ed.), 2011.*
18. *Lakić, I., Cincović, M. R., Belić, B., Đoković, R., Majkić, M., Petrović, M. Ž., Nikolić, S. Lipolysis and ketogenesis in cows in early lactation. Acta agriculturae Serbica 2018, 23(46), 265-276.*
19. *Benedet A., Manuelian C.L., Zidi A., Penasa M., De Marchi M.: Invited review: β-hydroxybutyrate concentration in blood and milk and its associations with cow performance. Animal 2019, 1-14.*
20. *Hachenberg S., Weinkauf C., Hiss S., Sauerwein H. Evaluation of classification modes potentially suitable to identify metabolic stress in healthy dairy cows during the peripartal period. Journal of Animal Science 2007, 85: 1923-1932.*
21. *Kessel S., Stroehl M., Meyer H.H.D., Hiss S., Sauerwein H., Schwarz F.J. Bruckmaier R.M. Individual variability in physiological adaptation to metabolic stress during early lactation in dairy cows kept under equal conditions. Journal of Animal Science 2008. 86: 2903-2912.*
22. *González F.D., Muiño R., Pereira V., Campos R., Benedito J.L. Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. Journal of Veterinary Science 2011, 12: 251-255.*
23. *Cincović M.R., Belić B., Radojičić B., Hristov S., Đoković R. Influence of lipolysis and ketogenesis to metabolic and hematological parameters in dairy cows during periparturient period. Acta veterinaria 2012, 62: 429-444.*
24. *Belić B., Cincović M., Lakić I., Đoković R., Petrović M., Ježek J., Starič J. Metabolic Status of Dairy Cows Grouped by Anabolic and Catabolic Indicators of Metabolic Stress in Early Lactation. Acta Scientiae Veterinariae 2018, 46(1):9.*
25. *Walton R.M. Subject‐based reference values: biological variation, individuality, and reference change values. Veterinary clinical pathology 2012, 41(2), 175-181.*
26. *Fraser C.G., Harris EK. Generation and application of data on biological variation in clinical chemistry. Critical Reviews in Clinical Laboratory Sciences 1989, 27:409–437.*
27. *Fraser C.G. Inherent biological variation and reference values. Clinical Chemistry and Laboratory Medicine 2004, 42:758–764.*
28. *Fraser C.G. Reference change values.Clinical Chemistry and Laboratory Medicine 2012, 50, 807-812.*
29. *Lund F., Petersen P.H., Fraser C.G. Different percenteges of false-positive results obtained using five methods for the calculation of reference chanfe values based on simulated normal and in-normal distributions of data. Annals of Clinical Biochemistry 2016, 53, 692-698.*
30. *Wayne P.A.: CLSI: Use of delta checks in the medical laboratory, Approved Guideline, Firs Edition, CLSI Document EP33, Clinical Laboratory Standards Institute, 2016.*
31. *Strathamann F.G:, Baird G.S., Hoffman N.G. Simulations of delta check rule performance to detect specimen mislabeling using historical laboratory data. Clinica Chimica Acta 2011, 412,1973-1977.*
32. *Zhang P., Tang H., Chen K. Biological variations of hematologic parameters determinated by UniCel DxH 800 hematology analyzer. Archives of Pathology and Laboratory Medicine 2013, 137:1106-1110.*
33. *Sanchez-Navarro L., Castro-Castro M.J.,Dot-Bach D., et al. Estimation of alert and change limits of haematologies quantities and its application in the plausibility control. EJIFCC 2014, 25:115-127.*
34. *Dunn E.J., Moga P.J. Patient misindentification in laboratory medicine: a qualitative analysis of 227 root cause analysis reports in the Veterans Health Administration. Archives of Pathology and Laboratory Medicine 2010, 134:244-255.*
35. *Schifman R.B.,Talbert M., Souers R.J. Delta check practices and outcomes: a Q-probes study involving 49 health care faclities and 6541 delta check alerts. Archives of Pathology and Laboratory Medicine 2017, 141: 813-823.*
36. *Ford A. Delta checks as a safety net: how used, how useful. CAP Today 2015, 29: 22-24.*
37. *Cincović M.R.(ur.), Starič J. (ur.). Laboratorijska istraživanja metaboličkog statusa goveda/ Laboratorijske raziskave pesnovnega stanja goveda. Monografija, Novi Sad/Ljubljana 2017.*
38. *Clinical and laboratory standards institute (CLSI). Use of Delta Checks in the Medical Laboratory. 2nd ed. CLSI guideline EP33. Clinical and Laboratory Standards Institute; 2023.*

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Review
Pregledni rad

FREKVENCIJA MUŽE I NJEN UTICAJ NA POIZVODNE I METABOLIČKE KARAKTERISTIKE KRAVA

MILKING FREQUENCY AND ITS INFLUENCE ON DERIVATIVE AND METABOLIC CHARACTERISTICS OF COWS

Srđan Krnjaić[a*], Radojica Djoković[b], Miloš Ž. Petrović[b], Mira Majkić[a], Jože Starič[c]

[a]Departman za veterinarsku medicinu, Poljoprivredni fakultet Novi Sad, Srbija
[b]Agronomski fakultet Čačak, Srbija
[c]Veterinarski fakultet Ljubljana, Slovenija
*Autor za korespodenciju: srdjankrnjaic66@gmail.com

R.DJ. https://orcid.org/0000-0003-0900-3227, M.Ž.P. https://orcid.org/0000-0002-4707-6796, M.M. https://orcid.org/0009-0007-0110-7327, J.S. https://orcid.org/0000-0002-5156-5277

SAŽETAK

Povećanje učestalosti muže može ad poveća proizvodnju mleka kod krava za 15-20%, a povećana frekvencija muže može uticati na različite mehanizme sekretone i metaboliček aktivnosti vimena. Krave se u praksi najlešće muzu dva puta dnevno sa preosečnim razmakom između dve muže od 12 sati, a u praksi je to najčešći raspon od 8-16 sati. Pokazano je da razmak od 12 časova između dve muže je bio dobar kod visokoproduktivnih krava, sa stabilnim rastom u proizvodnji mleka tokom prve polovine laktacije. Produžetak interval između dve muže dovešće do inhibicije laktacije sa posledičnim opadanjem količine proizvedenog mleka. Povećanje učestalosti muže mlečnih goveda na više od dve muže dnevno rezultira povećanjem proizvodnje mleka. Sprovođenje muže tri puta dnevno dovodi do povećanja proizvodnje mleka od 3 do 39% u poređenju sa dvokratnom mužom. Jedan od najočiglednijih efekata kao rezultat povećane učestalosti muže je mobilizacija telesnih rezervi. Primarni mehanizam koji dovodi do ovoga je lipoliza. Kao rezultat lipolize dolazi do porasta koncentracije NEFA i BHB u krvnoj plazmi odnosno serumu uz opadanje ocenjene telesne kondicije krava, a navedene promene se smatraju tipičnim odgovorom na povećanje frekvencije muže. U ogledima je pokazano značajno opadanje ocene telesne kondicije kod krava koje se muzu šestokratno u odnosu na trokratno mužene krave. Povećana lipoliza i ketogeneza dovode do metaboličkog odgovora i adaptiranja krava, a te promene nekada mogu dovesti do povećanja rizika za nastanak ili pak povećanu prevalenciju metaboličkih bolesti u stadima krava.

Ključne reči: krave, frekvencija muže, proizvodnja mleka, metabolička adaptacija.

ABSTRACT

Increasing milking frequency can increase milk production in cows by 15-20%, and increased milking frequency can affect different mechanisms of secretion and metabolic activity of the udder. In practice, cows are best milked twice a day with an average interval between two milkings of 12 hours, and in practice it is the most common range of 8-16 hours. It was shown that a 12-hour interval between two milkings was good for high-yielding cows, with a steady increase in milk production during the first half of lactation. An extended interval between two milkings will lead to inhibition of lactation with a consequent decrease in the amount of milk produced. Increasing the milking frequency of dairy cattle to more than two milkings per day results in an increase in milk production. Milking three times a day leads to an increase in milk production of 3 to 39% compared to milking twice. One of the most obvious effects as a result of increased milking frequency is the mobilization of body reserves. The primary mechanism leading to this is lipolysis. As a result of lipolysis, there is an increase in the concentration of NEFA and BHB in the blood plasma or serum with a decrease in the assessed body condition of the cows, and the mentioned changes are considered a typical response to an increase in milking frequency. The tests showed a significant decrease in the body condition score in cows that were milked six times compared to cows that were milked three times. Increased lipolysis and ketogenesis lead to a metabolic response and adaptation of cows, and these changes can sometimes lead to an increase in metabolic diseases in cow herds.

Key words: cow, milking frequency, milk production, metabolic adaptation.

FREKVENCIJA MUŽE KAO ZNAČAJAN FAKTOR U POSTIZANJU BOLJE PROIZVODNJE MLEKA

Povećana proizvodnja mleka za sobom povlači selekciju krava koje imaju određene osobine laktacione krive, ali i morfološke osobine vimena. Dobro struktuisano i veliko vime omogućuje visoku proizvodnju mleka, a zaključeno je da maksimalni potencijal krave mogu ispoljiti pomoću tehnoloških postupaka kao što je povećana frekvencija muže ili u novije vreme uvođenje muznih robota. Ipak, da bi se povećala proizvodnja na adekvatan način potrebno je bilo proučiti veze između stvaranaj mleka, lučenja mleka, morfoloških osobina vimena i optimalnog rasporeda muže (1-11). Povećanje učestalosti muže može ad poveća proizvodnju mleka kod krava za 15-20%, a povećana frekvencija muže može uticati na različite mehanizme sekretone i metaboliček aktivnosti vimena. Krave se u praksi najlešće muzu dva puta dnevno sa preosečnim razmakom između dve muže od 12 sati, a u praksi je to najčešći raspon od 8-16 sati. U prvim istpivanjima vršeno je da li vremenski intervali između dve muže mogu uticati na proizvodnju mleka. Pokazano je da razmak od 12 časova između dve muže je bio dobar kod visokoproduktivnih krava, sa stabilnim rastom u proizvodnji mleka tokom prve polovine laktacije. Međutim promena intervala muže, tačnije produžetak dovešće do inhibicije laktacije sa posledičnim opadanjem količine proizvedenog mleka. Pokazalo se da postoji veliko intraindividualno variranje kada je u pitanju interval između dve muže koji neka jedinka može da toleriše a da ne dođe do inhibicije produktivnosti usled promene intervala. Tada se shvatilo da je potrebno birati one kraev koje imaju velike cisterne vimena i koje mogu da skladište velike količine mleka. Danas postoji veći broj tehnologija za snimanje kapaciteta cisterne vimena kako bi se izvršilo objektivno merenje i odabir najkvalitetnijih jedinki. Što je cisterna vimena veća, proizvodiće se veća količina mleka, uz veću teoleranciju promene frekvencije muže. Sa druge strane kod životinja koje imaju male cistern, one ne mogu da prenseu alveolarno mleko tako lako, kada se stvara refleksna kočnica sekrecije mleka, a proizvodnja mleka lako opada kod promenljivog interval između dve muže. Eksperimentom je pokazano da što su uslovi za neprekidno isticanje mleka iz alveolarnog delja bolji, to je proizvodnja mleka veća. Zbog toga je povećanje frekvencije muže jedan od osnovnih načina za povećanje proizvodnje mleka.

Povećanje učestalosti muže mlečnih goveda na više od dve muže dnevno rezultiralo je povećanjem proizvodnje mleka (12-21). Sprovođenje muže tri puta dnevno dovodi do povećanja proizvodnje mleka od 3 do 39% u poređenju sa dvokratnom mužom kod krava muzara, 15-35% kod ovaca i 10-20% kod koza. Najveći prinosi pri primeni trokratne muže postojali su kod visokoproduktivnih primiparnih krava u odnosu na multiparne, a gledano sa aspekta perioda laktacije najveći prinosi se dobijaju prilikom aplikacije trokratne muže u kasnijim fazama laktacije. Veliki broj istraživanja je pokazao da prelazak sa dvokratne na trokratnu mužu rezultira stabilnim povećanjem mleka od oko 3,5 kg po danu, a da taj porast uglavnim nije proporcionalno povećan. Trokratna muža je, vrlo verovatno, biološki optimalna frekvencija muže, jer je pokazano da krave koje su držane slobodno i koje su samoinicijativno prilazile robotu za mužu su to činile od 2,7 do 3,9 puta na dan. Povećanje frekvencije muže će dovesti do povećane proizvodnje mleka u zavisnosti od toga koliko dugo se primenjuje određena frekvencija muže. Tako recimo kod primene četvorokratne muže u period od mesec dana primećen je porast proizvodnje mleka od 14%, prelazak na šestokratnu mużu dovešće do porasta od 10 do 15% u roku od samo dva dana. Navedeni podaci ukazuju da povećanje frekvencije muže na 4 putadnevno omogućuje dosta visok, a lagan porast porast, dok dalje povećanje frekvencije daje porast ali ne toliko veliki kao kod četvorokratne muže. Ovo je potvrđeno kako kod krava, tako i kod malih preživara. Pored povećanja količine proizvedenog mleka postoje i promene u sastavu mleka ali su nalazi vezani za sastav mleka

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kontradiktorne. Pojedini autori su našli minorne i statistički nesignifikantne promene u biohemijskom sastavu mleka, dok su pojedini istraživači našli smanjenje mlečne masti u odnosu na standardnu dvokratnu mužu. Takođe rezultati su kontradiktorni i sa aspekta činjenice da li su promene izraženije kod krava koje su primirparne ili multiparne. Primećen je blagi pad proteina i kazeina u mleku koji je bio toliki da je prinos sira od tog mleka bio manji za oko 1,5%. Broj somatskih ćelija kao indicator zdravlja vimena i mikrobiološkog kvaliteta mleka je opadao sa povećanjem frekvencije muže. Dakle povećanje frekvencije muže može da poboljša zdravlje vimena i higijenske parametre mleka. Povećanje frekvencije muže stimuliše sekreciju laktogenih hormona koji dalje omogućavaju bolju perzistenciju laktacije, jer pomenuti hormone povećavaju broj sekretornih ćelija i samim tim zapreminu izlučenog mleka. Involucija vimena uz apoptozu ćelija se biološki depava sa napredovanjem laktacije, ali se aktivnost preostalih ćelija održava na visokom nivou, a smanjenje broja ćelija je modifikovano učestalošću muže. Takođe, povećanjem frekvencije muže povećava se proliferativni kapacitet novih ćelija, a nađena je i povećana enzimatska aktivnost ćelija što ukazuje na povećanu spremnost ćelija na novu sintezu mleka, što je dokazano kod krava i koza.

Posledice smanjenja broja muža na proizvodnju mleka preživara proučavali su mnogi autori (22-31). Prelazak sa dvokratne na jednokratnu mužu ndovodi do opadanja proizvodnje mleka u rasponu od 10 do čak 50% kod mlečnih krava. Čak i kratkotrajni prelazak sa dve muže na jednu dovelo je do opadanja proizvodnje mleka za 10-25% kod Holštajn-frizijskih krava koje su bule u sredini laktacije. Istraživanja su potvrdila da je period laktacije u kom se smanjuje frekvencija muže veoma bitna, pa tako količina mleka opada za 38% u ranoj laktaciji, odnosno za 28% u kasnoj laktaciji. Ovakav nalaz je logičan, jer u ranoj laktaciji proizvodnja mleka mnogo više zavisi od različitih faktora spoljašnje sredine u odnosu na kasnije periode laktacije. Promene u količini proizvedenog mleka zavisiće od udela mleka u mlečnoj žlezdi, odnosno od kapaciteta cistern da prihate mleko. Istraživanja sprovedena na ovcama pokazuju da ovce sa velikim kapacitetom skladištenja mleka dolazi do mnogo manjih gubitaka u proizvodnji mleka, u odnosu na ovce koje sui male slab kapacitet skladištenja mleka, što je dobijeno poređenje rase ovaca sa sardinije ili kod kanarskih koza koje imaju veliki kapacitet cistern i nekih alpskih rasa ovaca ili pojedinih rasa koza sa malim kapacitetom rezervoara. Na osnovu svega zaključujemo da je smanjenje učestalost muže uzrok opadanja proizvodnje mleka, a da to zavisi od skladišnog kapaciteta životinje. Sa smanjenjem frekvencije muže češće dolazi do porasta broja somatskih ćelija, dok promene u hemijskom sastavu mleka sa aspekta proteina i masti najčešće nisu značajne, osim kod koza gde je zabeležen porast mlečne masti i kazeina u mleku posle smanjenja frekvencije muže. Postoji nekoliko značajnih mehanizama koji se aktiviraju sa smanjenjem frekvencije muže, odnosno sa povećanjem vremena između dve muže i oni uključuju: povećanje alveolarne distenzije, smanjenje protoka krvi u vimenu, povećanje propustljivosti čvrstog spoja i akumulacija inhibitora laktacije.

DVOKRATNA I TROKRATNA MUŽA I METABOLIČKA ADAPTACIJA KRAVA U RAZLIČITIM PERIODIMA LAKTACIJE

U cilju postizanja visoke proizvodnje mleka i maksimiziranja potencijala do kojih se dolazi genskom selekcijom uvođenje tehnoloških manipulacija koje znače povećanje frekvencije muže deluje opravdano. Veliki broj istraživanja pokazao je kako prelazak sa dvokratne na trokratnu mužu utiče na produktivnost kod krava (32-42). Kod krava muženih trokratno dobija se povećanje proizvodnje mleka od 1,72 kg po danu u odnosu na iste krave koje su mužene dvokratno. Ovakvo povećanje u proizvodnji melka dovelo je opadanja telesne kondicije tako da je mlečna kondicija bila još izraženija. Povećao se i prinos proteina u mleku za oko 0,15 kg/dan kod trokratno muženih krava u odnosu na dvokratno mužene jedinke, a on prati povećanu proizvodnju mleka. U navedenim istraživanjima porasla je i proizvodnja mlečne masti kod trokratno pomuženih krava. Povećanje prinosa masti u jendom ogledu zavisilo je od povećanja prinosa mleka posle aplikacije trokratne muže na jedinke koje se mzu dvokratno. Tako je povećanje prinosa mleka od 3,46 kg/dan dovelo do povećanja prinosa masti od 0,05 kg/dan, a kada je povećanje prinosa pomuženog mleka bilo3,69 kg/dan postojalo je mnogo veće povećanje mlečne masti od 0,11 kg/dan. Međutim, ostaje otvoreno pitanje na koji način frekvencija muže utiče na unos hrane i da li povećanje frekvencije muže može koje prati povećane prinose mleka i njegovih ingredijenata možeda bude ispraćeno adekvatnim unosom hrane ili se povećavaju homeoretski mehanizmi. Ovo je

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pitanje koje se našlo u okviru našeg istraživanja, gde će energetski bilans krava koji je povezan sa unosom hrane biti ispitan kao poseban faktorijel kod krava u dvokratnoj i trokratnoj muži i u različitim periodima laktacije. U jednoj velikoj meta-analizi pokazano je da postoji umerena heterogenost u promeni vrednosti mlečnih proteina i masti, a nađena j visoka heterogenost kada se radi o unosu suve materije hrane. Kada se izvrši pristranost analiza, nije nađena pristrasnost za ispitivane parametre, ali su autori primetili postojanje blage pristrasnosti što ukazuje da, sagledano sa aspekta velikog broja istraživanja, postoji povezanost proizvodnje mleka i mlečne masti u funkciji frekvencije muže.

Jedan od najočiglednijih efekata kao rezultat povećane učestalosti muže je mobilizacija telesnih rezervi (43-47). Već smo napomenuli ranije da sa porastom frekvencije muže opada telesna kondicija. Primarni mehanizam koji dovodi do ovoga je lipoliza. Kao rezultat lipolize dolazi do porasta koncentracije NEFA i BHB u krvnoj plazmi odnosno serumu uz opadanje ocenjene telesne kondicije krava, a navedene promene se smatraju tipičnim odgovorom na povećanje frekvencije muže. U ogledima je pokazano značajno opadanje ocene telesne kondicije kod krava koje se muzu šestokratno u odnosu na trokratno mužene krave. Suprotno, sa opadanjem frekvencije muže nađen je porast telesne kondicije kao i njeno slabije opadanje, što je posebno izraženo kod primene muže jednom dnevno. Promene u metabolizmu koje nastaju sa povećanjem frekvencije muže predstavljaju sastavni deo složenih homeoretskih prestrojavanja koje smo ranije opisali. Metaboličke promene zavise od od koordinacije perifernih signala, hranljivih materija i hormona, sa lokalnim regulatornim mehanizmima koji prilagođavaju stope dostupnosti hranljivih materija tako da odgovaraju sintetičkim kapacitetima epitelnih ćelija mlečne žlezde, što zapravo predstavlja homeorezu. Tako je kod krava koje se muzu višekratno nađena povećana koncentraciaj hormona rasta uz porast koncentracije prolaktina i oksitocina, a opadala je vrednost insulina. Hormonski status krava potvrđuje da povećanje frekvencije muže (u ogledu u kom je merena koncentracija hormona radio se o šestokratnoj muži dnevno) dovodi do tipičnog endokrinološkog prestrojavanja karakterističnog za homeorezu koja se vidi prilikom prelaska iz perioda zasušenja u period laktacije. Kod ispitivanih krava nađen je porast vrednosti IGF-I, što ukazuje na anaboličke efekte hormona rasta na periferna tkiva. Pored sistemskih efekata i promena, postoje i promene u samoj mlečnoj žlezdi, gde je nađen porast aktivnosti različitih enzima uz porast sinteze laktoze kao i genskog materijala koji učestvuje u formiranju korisnih enzima za produkciju mleka. Poređenjem metaboličkih parametara kod krava koje se muzu šest puta dnevno u odnosu na trokratno mužene krave nađena je značajno viša koncentracija BHB i NEFA ali i niža koncentracija glukoze. Iako se ovde radi o dosta velikom povećanju frekvencije muže, gotovo identični rezultati su dobijeni kada se prelazi sa jednokratne na dvokratnu mužu. U oba slučaja postojalo je povećane prinosa mleka koji nije bio adekvatno ispraćen povećanjem energetskog unosa poreklom iz hrane.

Na osnovu svega navedenog zaljučujemo da lipoliza i ketogeneza predstavljaju značajne mehanizme prestrojavanja celokupnog metabolizma krava prilikom započinjanja laktacije, pa je sagledavanje veze metabolita sa parametrima lipolize i ketogeneze najpogodniji način da se utvrdi na koji način povećana frekvencija muže utiče na metaboličku adaptaciju kod krava. Rezultati naših istraživanja (48) pokazuju sledeće:

Frekvencija muže je imala statistički značajan uticaj na niovu $p< 0,01$, osim za ALB gde je uticaj utvrđen naivou $p< 0,05$ za sledeće ispitivane parametre NEFA, BHB, GLU, TBIL, AST, GGT, Ca, ALB, INS, T4 i kortizol (CORT). Srednje vrednosti parametara kod krava u dvokratnoj u odnosu na krave u trokratnoj muži iznosile su: NEFA (0,45: 0,66 mmol/L), BHB (0,47: 1,16 mmol/L), GLU (2,66:2,38 mmol/L), TBIL (6,82: 9,54 μmol/L), AST (34,5: 48,8 U/L), GGT (11,5: 16,2 U/L), Ca (2,63: 2,13 mmol/L), ALB (32,35: 29,96 g/L), INS (4,91: 4,15 mU/L), T4 (38,09: 28,64 nmol/L) i CORT (12,78: 18,32 nmol/L). Rezultati ispitivanja pokazuju da kod krava u trokratnoj muži postoji viša koncentracija NEFA, BHB, TBIL, AST, GGT i CORT, a niža koncentracija GLU, Ca, ALB, INS i T4 u odnosu na krave u dvokratnoj muži.

Period laktacije je pokazao značajan uticaj na najveći broj ispitivanih parametara na nivou $p<0,01$ i to za NEFA, BHB, GLU, CHOL, TGC, TBIL, AST, GGT, TPROT, ALB, UREA, INS, RQUICKIBHB, T3, T4 i kortizol (CORT). Dinamika u promeni izmerenih koncentracija u sva tri perioda laktacije od rane ka stabilnoj za parametre kod kojih je utvrđena statistički signifikantna značajnost podrazumevala je sledeće: NEFA (0,83: 0,44: 0,39 mmol/L), BHB (1,12: 0,7: 0,62 mmol/L), GLU (2,21: 2,58: 2,8 mmol/L), CHOL (3,23: 3,85: 4,26 mmol/L), TGC

(0,12: 0,14: 0,15 mmol/L), TBIL (11,71: 7,52: 5,3 μmol/L), AST (59,69: 38,4: 26,84 IU/L), GGT (19,9: 12,8: 8,96 IU/L), TPROT (65,85: 70,0: 71,53 g/L), ALB (26,89: 32,7: 33,87 g/L), UREA (5,86: 4,63: 4,52 mmol/L), INS (3,71: 4,83: 5,05 mU/L), RQUICKIBHB (0,51: 0,63: 0,62), T3 (0,79: 1,05: 1,04 nmol/L), T4 (26,16: 35,59: 38,35 nmol/L) i CORT (18,28: 13,86: 14,51 nmol/L). Na osnovu rezultata zaključujemo da je koncentracija NEFA, BHB, TBIL, AST, GGT, UREA i CORT viša u ranoj laktaciji a da potom opada, dok je vrednost TPROT, ALB, INS, RQUICKIBHB, T3 i T4 niža u ranoj laktaciji a da potom postepeno raste.

Od velikog značaja je interakcija frekvencije muže i perioda laktacije. Signifikantan uticaj interakcije ova dva faktora ukazuje na to da oba faktora paralelno pokazuju uticaj na metaboličku adaptaciju karva. Interakcija frekvencije muže i perioda laktacije značajno utiče na vrednosti NEFA i BHB, tako da kod krava u trokratnoj muži postoji viša vrednost ovih parametara, koja je još više raste u ranoj laktaciji u odnosu na krave u dvokratnoj muži. Sličnu dinamiku pokazali su TBIL kao i enzimi jetre AST i GGT.

Najveći broj ispitivanih metaboličkih parametara pokazuje mnogo veću povezanost sa NEFA u trokratnoj muži u odnosu na dvokratnu. To znači da su vrednosti i varijabilnost ispitivanih parametara mnogo više determinisani vrednostima NEFA (viša vrednost koeficijenta determinacije R^2) kod krava u trokratnoj u odnosu na dvokratnu mužu. Koeficijenti determinacije u trokratnoj i dvokratnoj muži imali su sledeće vrednosti za ispitivane parametre: GLU (0,63: 0,074), CHOL (0,292: 0,016), TGC (0,29: 0,022), TBIL (0,666: 0,022), AST (0,666: 0,02), Mg (0,132: 0,032), TPROT (0,234: 0,007), ALB (0,364: 0,002), UREA (0,243: 0,046), INS (0,35: 0,017), RQUICKIBHB (0,355: 0,082), T3 (0,312: 0,000), T4 (0,187: 0,004) i CORT (0,272: 0,026). Pored navedenog, vrednost regresionog parametra b ukazuje da promena vrednosti svakog od ispitivanih metabolita po jedinici NEFA je mnogo izraženija kod krava u trokratnoj u odnosu na dvokratnu mužu, što ukazuje da postoji veća osetljivost krava na lipolizu ukoliko se poveća frekvencija muže.

Korelaciona i regresiona analiza pokazuje da postoji razlika u metaboličkoj adaptaciji na ketogenezu, odnosno vrednost BHB u zavisnosti od toga da li se krave muzu dvokratno ili trokratno, što je prikazano na grafikonima 19-36. Koeficijent determinacije između ispitivanih parametara i BHB bio je viši kod krava u trokratnoj muži: GLU (0,639: 0,082), CHOL (0,453: 0,046), TGC (0,502: 0,054), TBIL (0,549: 0,215), AST (0,565: 0,217), GGT (0,565: 0,217), P (0,128:0,001), Mg (0,179: 0,039), TPROT (0,430: 0,036), ALB (0,356: 0,011), UREA (0,251: 0,132), INS (0,375: 0,079), RQUICKIBHB (0,379: 0,029), T3 (0,240: 0,058), T4 (0,204: 0,023) i CORT (0,442: 0,053). Promena vrednosti metaboličkih parametara za svaku jedinicu BHB odnosno regresioni parametar b ukazuju da nije bilo velikog odstupanja u promeni koncentracije metabolita za svaku jedinicu BHB u trokratnoj u odnosu na dvokratnu mužu posmatrajući sa aspekta celokupne laktacije.

Dobijeni rezultati pokazuju da sa povećanjem frekvencije muže metabolička adaptacija kod krava u znatnijoj meri zavisi od lipolize i ketogeneze. Promena vrednosti metaboličkih parametara u trokratno u odnosu na dvokratnu mužu je mnogo veća sa porastom lipolize (vrednost NEFA) u odnosu na porast ketogeneze (vrednost BHB).

MF, EB, LP i MF×EB su pokazali značajne efekte na metaboličke parametre, dok drugi MF×LP i trosmerna interakcija MF×EB×LP nisu pokazali značajne efekte na većinu parametara krvi.

Na osnovu ispitivanih biohemijskih parametara krvi moguće je razlikovati krave u ranoj laktaciji u odnosu na srednju i kasnu laktaciju, delimično se razlikuju krave prema energetskom bilansu, ali nije moguće razlikovati krave u dvokratnoj i trokratnoj. Međutim, kada se krave klasifikuju na osnovu interakcije energetskog bilansa i muže dobijamo klastere, gde se najviše izdvaja klaster krava koje se muzu trokratno i nalaze se u negativnom energetskom bilansu, koji se delimično preklapa sa kravama u dvokratnoj muži i negativnom energetskom bilansu i ne poklapa se sa klasterima krava koje su u pozitivnom energetskom bilansu. Navedeni podaci ukazuj da trokratna muža i negativni energetski bilans dovode do značajnih metaboličkih odstupanja kod mlečnih krava.

Vrednost pojedinačnih krvnih parametara ispitana je u funkciji perioda laktacije, EB, frekvencije muže i interakcije frekvencije muže i energetskog bilansa. Period laktacije je uticao na sve ispitivane parametre osim na vrednosti Ca i P. U ranoj laktaciji postoji viša vrednosti NEFA, BHB, AST, GGT, TBIL, UREA i CORT, a niža vrednost GLU, CHOL, TGC, TPROT, ALB, INS, RQUICKIBHB, T3 and T4. Frekvencija muže je uticala na sve parametre osim na CHOL, TGC, P, TPROT, ALB i UREA. Kod krava u trokratnoj muži nađena je viša vrednost NEFA, BHB, AST, GGT, TBIL i CORT, a niža vrednost GLU, Ca, INS i T4. Energetski bilans je pokazao uticaj na sve ispitivane faktore, osim na Ca. Krave u negativnom energetskom bilansu pokazuju viša vrednosti NEFA, BHB, AST, GGT, TBIL, UREA i CORT, a niža vrednost GLU, CHOL, TGC, TPROT, ALB, P, INS, RQUICKI, T3 and T4. Interakcija frekvencija muže × energetski bilans je uticao na sve odabrane parametre osim na Ca, APB, UREA i T4. Nađeno je da kod krava u trokratnoj muži i negativnom energetskom bilansu postoji viša koncentracija NEFA, BHB, AST, GGT, TBIL i CORT, a niža vrednost GLU, CHOL, TGC, TPROT, P, INS, RQUICKIBHB i T3 u odnosu na krave u dvokratnoj muži i negativnom energetskom bilansu i krave u pozitivnom energetskom bilansu.

Efekti EB, NEFA i BHB na krvne parametre razlikovali su se u funkciji frekvencije muže i energetskog bilansa kod krava. Postoje vrlo jake korelacione veze kod krava u trokratnoj muži i negativnom energetskom bilansu, i potom opadaju tako da su nešto slabije kod krava u dvokratnoj muži i negativnom energetskom bilansu, još slabije kod krava u trokratnoj muži i pozitivnom energetskom bilansu, a mnoge veze se potpuno gase kod krava u dvokratnoj muži i pozitivnom energetskom bilansu. Poređenjem koeficijenata korelacije zaključujemo da EB, NEFA i BHB značajno više koreliraju sa metaboličkim parametrima kod krava u trokratnoj muži i negativnom energetskom bilansu (R=0,78-0,95) u odnosu na grupu dvokratna muža i negativni energetski bilans (R=0,56-0,79), kao i u odnosu na grupu trokratne muže (R=0,35-0,59) i dvokratne muže (R=0,06-0,31) koje su u pozitivnom energetskom bilansu. Negativni energetski bilans dovodi do značajno veće povezanosti metaboličkih parametara sa EB, NEFA i BHB kod krava u trokratnoj muži u odnosu na ostale ispitivane grupe. Određeni parametri, kao što su Ca i P ne pokazuju nikakvu vezu sa EB, NEFA i BHB bez obzira na grupu krava. Zaključujemo da kod krava u trokratnoj muži postoji mnogo izraženija lipoliza i ketogeneza (više vrednosti NEFA i BHB) sa opadanjem vrednsoti EB. U trokratnoj muži je ketogeneza mnogo intenzivnija (porast BHB) sa razvojem lipolize (porast NEFA).

Pored jačine veze od velikog značaja je određivanje nagiba regresione krive kako bi se utvrdilo koliko se metabolički parametri menjaju u funkciji vrednosti EB, NEFA i BHB kod krava u dvokratnoj i trokratnoj muži koje su u pozitivnom i negativnom energetskom bilansu. Relativni efekat EB, NEFA i BHB na parametre je različit i zavisi od grupe životinja i od parametra na koji deluju. Kod krava u negativnom energetskom bilansu vrednost GLU, CHOL, TGC, značajno više zavisi od vrednosti EB. Kod krava u trokratnoj muži i negativnom bilansu vrednosti TBIL, AST i GGT značajno više zavise od EB u odnosu na ostale grupe krava. Vrednost TPROT i ALB značajno više zavisi od EB kod krava u pozitivnom bilansu, dok vrednostu UREA više zavisi kod krava u negativnom energetskom bilansu. INS značajno više zavisi od EB kod krava u dvokratnoj muži i pozitivnom bilansu, dok vrednost T3 značajno više zavisi od EB samo kod krava u trokratnoj muži. RQUICKIBHB pozitivno odstupa sa porastom EB kod krava u negativnom bilansu. Kortizol opada sa porastom vrednosti EB, što je najizraženije kod krava u trokratnoj mužu i pozitivnom energetskom bilansu, dok nema efekta kod krava koje se muzu dvokratno i nalaze se u pozitivnom bilansu. Vrednosti GLU, CHOL, TGC, TPROT, ALB, INS, T3, T4 opadaju, a vrednosti AST, GGT, TBIL, UREA i CORT rastu sa porastom NEFA i BHB. Zavisnost TGC, TBIL, AST, GGT i CORT od NEFA je veća kod krava sa trokratnom mužom bez obzira na energetski bilans, dok je zavisnost GLU, UREA, INS i T4 od NEFA veća kod krava u negativnom energetskom bilansu bez obzira na frekvenciju muže. RQUICKIBHB više zavisi od NEFA kod krava u pozitivnom energetskom bilansu. Zavisnost odabranih krvnih parametara od BHB pokazuje ujednačenost bez obzira na energetski bilans i frekvenciju muže, s tim što zavisnost opada kod krava u dvokratnoj muži i pozitivnom energetskom bilansu u odnosu na druge grupe krava.

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LITERATURA

1. *Knight C.H., Hirst D., Dewhurst R.J. Milk accumulation and distribution in the bovine udder during the interval between milkings. The Journal of Dairy Research. 1994, 61:167-177*
2. *Davis S.R., Farr V.C., Copeman P.J.A., Carruthers V.R., Knight C.H., Stelwagen K. Partitioning of milk accumulation between cisternal and alveolar compartments of the bovine udder: Relationship to production loss during once daily milking. The Journal of Dairy Research 1998, 65:1-8*
3. *Ayadi M., Caja G., Such X., Knight C.H. Use of ultrasonography to estimate cistern size and milk storage at different milking intervals in the udder of dairy cows. The Journal of Dairy Research 2003, 70:1-7*
4. *Eslamizad M., Dehghan-Banadaky M., Rezayazdi K., Moradi-Shahrbabak M. Effects of 6 times daily milking during early versus full lactation of Holstein cows on milk production and blood metabolites. Journal of Dairy Science 2010, 93(9):4054-4061*
5. *Ruberte J., Carretero A., Fernandez M., Navarro M., Caja G., Kirchner F., et al. Ultrasound mammography in the lactating ewe and its correspondence to anatomical section. Small Ruminant Research 1994, 13:199-204*
6. *Caja G., Such X., Ruberte J., Carretero A., Navarro M. The use of ultrasonography in the study of mammary gland cisterns during lactation in sheep. In: Proceedings of the International Symposium on Milking and Milk Production of Dairy Sheep and Goats. Wageningen, The Netherlands: Wageningen Pers; 1999. pp. 91-93*
7. *Caja G., Salama O.A., Fathy A., El-Sayed H., Salama A.A.K. Milk partitioning and accumulation in the camel udder according to time elapsed after milking. In: Proceedings of the 62nd Annual Meeting of EAAP. Stavanger; 2011. p. 363*
8. *Castillo V., Such X., Caja G., Salama A.A., Albanell E., Casals R. Changes in alveolar and cisternal compartments induced by milking interval in the udder of dairy ewes. Journal of Dairy Science 2008, 91:3403-3411*
9. *Rovai M., Caja G., Such X. Evaluation of udder cisterns and effects on milk yield of dairy ewes. Journal of Dairy Science 2008, 91:4622-4629*
10. *Marnet P.G., McKusick B.C. Regulation of milk ejection and milkability in small ruminants. Livestock Production Science 2001, 70:125-133*
11. *Stelwagen K., Knight C.H., Farr V.C., Davis S.R., Prosser C.G., McFadden T.B. Continuous versus single drainage of milk from the bovine mammary gland during a 24 hour period. Experimental Physiology 1996, 81:141-149*
12. *Stelwagen K., Knight C.H. Effect of unilateral once or twice daily milking of cows on milk yield and udder characteristics in early and late lactation. The Journal of Dairy Research 1997, 64:487-494*
13. *Peaker M., Blatchford D.R. Distribution of milk in the goat mammary gland and its relation to the rate and control of milk secretion. The Journal of Dairy Research 1988, 55:41-48*
14. *Stelwagen K. Effect of milking frequency on mammary functioning and shape of the lactation curve. Journal of Dairy Science 2001, 84, E204-E211*
15. *Klei L.R., Lynch J.M., Barbano D.M., Oltenacu P.A., Lednor A.J., Bandler D.K. Influence of milking three times a day on milk quality. Journal of Dairy Science, 1997, 80:427-436*
16. *Erdman R.A., Varner M. Fixed yield responses to increased milking frequency. Journal of Dairy Science 1995, 78:1199-1203*
17. *Ipema A.H. Integration of robotic milking in dairy housing systems review of cow traffic and milking capacity aspects. Computers and Electronics in Agriculture 1997, 17:79-94*
18. *Knight C.H., Wilde C.J. Mammary cell changes during pregnancy and lactation. Livestock Production Science 1993, 35:3-19*
19. *Allen D.B., DePeters E.J., Laben R.C. Three times a day milking: Effects on milk production, reproductive efficiency and udder health. Journal of Dairy Science 1986, 69:1441-1446*
20. *Knight C.H., Foran D., Wilde C.J. Interactions between autocrine and endocrine control of milk yield; thrice-daily milking of bromocriptine-treated goats. Journal of Reproduction and Fertility 1990, 5. Abstract. no. 30*
21. *Alshaikh M.A., Salah M.S. Effect of milking interval on secretion rate and composition of camel milk in late lactation. The Journal of Dairy Research 1994, 61:451-456*
22. *Davis S.R., Farr V.C., Stelwagen K. Regulation of yield loss and milk composition during once-daily milking: A review. Livestock Production Science 1999, 59:77-94*
23. *Molenaar A., Leath S.R., Caja G., Henderson H.V., Cameron C., Challies M., et al. Development of ultrasound methodology to measure cow udder cistern storage capacity in the new Zeland pasture-fed context. Proceedings of the New Zealand Society of Animal Production 2013, 73:114-116*
24. *Stelwagen K., Knight C.H., Farr V.C., Davis S.R., Prosser C.G., McFadden T.B. Continuous versus single drainage of milk from the bovine mammary gland during a 24 hour period. Experimental Physiology 1996, 81:141-149*

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25. *Ayadi M., Hammadi M., Khorchani T., Barmat A., Atigui M., Caja G. Effects of milking interval and cisternal udder evaluation in Tunisian Maghrebi dairy dromedaries (Camelus dromedarius L.). Journal of Dairy Science 2009, 92:1452-1459*
26. *Salama A.A.K., Such X., Caja G., Rovai M., Casals R., Albanell E., et al. Effects of once versus twice daily milking throughout lactation on milk yield and milk composition in dairy goats. Journal of Dairy Science 2003, 86:1673-1680*
27. *Wilde C.J., Knight C.H., Addey C.V.P., Blatchford D.R., Travers M., Bennett C.N., et al. Autocrine regulation of mammary cell differentiation. Protoplasma 1990, 159:112-117*
28. *Stelwagen K., Knight C.H. Effect of unilateral once or twice daily milking of cows on milk yield and udder characteristics in early and late lactation. The Journal of Dairy Research 1997, 64:487-494*
29. *Dewhurst R.J., Knight C.H. Relationship between milk storage characteristics and the short-term response of dairy cows to thrice-daily milking. Animal Production 1994, 58:181-187*
30. *Négrao J.A., Marnet P.G. Cortisol, adrenalin, noradrenalin and oxytocin release and milk yield during first milkings in primiparous ewes. Small Ruminant Research 2003, 67:69-75*
31. *Nudda A., Pulina G., Vallabell R., Bencini R., Enne G. Ultrasound technique for measuring mammary cistern size of dairy ewes. The Journal of Dairy Research 2000, 67:101-106*
32. *Erdman R.A., Varner M. Fixed yield responses to increased milking frequency. J Dairy Sci 1995, 78:1199-203.*
33. *Economides S. The effects of milking cows three times daily on milk yield, milk composition, and profitability compared to two times daily milking. Report. Agricultural Research Institute Ministry of Agriculture, Natural Resources and the Environment 1999, 3-12.*
34. *Allen D.B., DePeters E.J., Laben R.C. Three times a day milking: effects on milk production, reproductive efficiency, and udder health. J Dairy Sci 1986, 69:1441-6.*
35. *Atashi H. Effect of milking frequency on the lactation performance and lactation curve of Holstein dairy cows in Iran. Iranian J Appl Anim Sci 2015, 5:273-8.*
36. *Wall E.H., McFadden T.B. Use it or lose it: Enhancing milk production efficiency by frequent milking of dairy cows. J Anim Sci 2008, 86 Suppl 1:27-36.*
37. *Sirohi A.S., Pandey H.N., Singla M. Effects of milking frequency on feed intake, body weight and haemato-biochemical changes in crossbred cows. J App Anim Res 2012, 40:63-8.*
38. *Hart K.D., McBride B.W., Duffield T.F., DeVries T.J. Effect of milking frequency on behavior and productivity of lactating dairy cows. J Dairy Sci 2013, 96:6973-85.*
39. *Waterman D.F., Harmon R.J., Hemken R.W., Langlois B.E. Milking frequency as related to udder health and milk production. J Dairy Sci 1983, 66:253-8.*
40. *Amos H.E., Kisier T., Loewenstein M. Influence of milking frequency on productive and reproductive efficiencies of dairy cows. J Dairy Sci 1985, 68:732-9.*
41. *Pearson R.E., Fulton L.A., Thompson P.D., Smith J.W. Three times a day milking during the first half of lactation. J Dairy Sci 1979, 62:1941-50.*
42. *Poole D. The effects of milking cows three times daily. Animal Science 1982, 34:197-201. doi:10.1017/S0003356100000672*
43. *Soberon F., Lukas J.L., Van Amburgh M.E., Capuco A.V., Galton D.M., Overton T.R. Effects of increased milking frequency on metabolism and mammary cell prolif-eration in Holstein dairy cows. J Dairy Sci 2010, 93:565-73.34.*
44. *Roche J.R., Dillon P.G., Stockdale C.R., Baumgard L.H., Van Baale J.M. Relation-ships among international body condition scoring systems. J Dairy Sci 2004, 87:3076-9.35.*
45. *Bequette B.J., Douglass L.W. The frequency of unilateral milking alters leucine metabolism and amino acid removal by the mammary gland of lactating goats. J Dairy Sci 2010, 93, 162-169.*
46. *Bar-Pelled U., Maltz E., Bruckental I., Robinson B., Voet H., Tagari H. Relationship between frequent milking in early lactation and milk production of high produc-ing dairy cows. J Dairy Sci 1995, 78:2726-36.*
47. *Hillerton J.E., Knight C.H., Turvey A., Wheatley S.D., Wilde C.J. Milk yield and mamma-ry function in dairy cows milked four times daily. J Dairy Res 1990, 57:285-94*
48. *Krnjaić S., Cincović M., Djoković R., Belić B., Ježek J., Starič J. The influence of energy balance, lipolysis and ketogenesis on metabolic adaptation in cows milked twice and three times daily. Metabolites 2022, 12(11), 1090.*

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Review
Pregledni rad

SEVERE ADVERSE IMPACT OF BOVINE VIRAL DIARRHEA ON CATTLE PRODUCTION: A COMPREHENSIVE APPROACH TO CONTROL

ŠTETNI UTICAJ BOVINE VIRUSNE DIJAREJE NA PROIZVODNE KARAKTERISTIKE GOVEDA: SVEOBUHVATAN PRISTUP KONTROLI

Vladimir S. Kurćubić[a*], Marko Dmitrić[b], Saša Živković[b], Miloš Ži. Petrović[a]

[a]Faculty of Agronomy in Čačak, University in Kragujevac, Cara Dušana 34, 32102 Čačak, Serbia
[b]Veterinary Specialized Institute „Kraljevo“, Žička 34, 36000 Kraljevo, Serbia
*Corresponding author: vkurcubic@mts.rs

V.K. https://orcid.org/0000-0003-2016-7345, M.D. https://orcid.org/0000-0002-3510-696X, S.Ž. https://orcid.org/0000-0002-7352-4361, M.Ži.P. https://orcid.org/0000-0002-4707-6796

ABSTRACT

Bovine viral diarrhea (BVD) is caused by a pestivirus known as BVDV and is one of the most important infectious diseases of cattle, with a huge economic impact worldwide. The most important source of infection are persistently infected (PI) and diseased cattle. In addition to cattle infection, BVDV infection has been diagnosed in sheep, goats, pigs and wild ruminants (roe deer, deer, bison), as reservoirs of the virus and sources of infection in cattle herds. The consequences of BVDV infections are abortions in pregnant animals, poor female conception, mummification and congenital malformations of fetuses, respiratory problems, transplacental infections and fetal death, neonatal and postnatal mortality, mucosal diseases, slowed growth and poorer performance of surviving animals. Hemorrhagic syndrome (with thrombocytopenia and hemorrhage) is caused exclusively by non-cytopathogenic (NCP) BVDV genotype 2, i.e. virulent strains. The basis of the control program is the prevention of intrauterine infection by identifying and removing PI animals from the cattle herd. The high prevalence of BVDV in cattle worldwide and in Serbia is a danger and causes extremely high economic losses, preventing international trade in breeding and fattening cattle with EU countries, primarily due to uneven approaches or lack of control programs. There are suggestions that the control of the disease in Serbia could be based on a voluntary BVDV eradication program at the herd level, based on four phases, which includes frequent diagnostic tests, removal of PI animals from the herd and introduction of strict biosecurity measures. Certification and register of herds with BVDV free status could be done through the Veterinary Administration.

Key words: Cattle, Bovine viral diarrhoea, Pestivirus, BVDV, Prevalence, Disease control, Economic impact

SAŽETAK

Bovina virusna dijareja (BVD) je uzrokovana pestivirusom poznatim kao BVDV i jedna je od najvažnijih zaraznih bolesti goveda, sa ogromnim ekonomskim uticajem širom sveta. Najvažniji izvor infekcije su perzistentno zaražena (PI) i bolesna goveda. Pored infekcije goveda, BVDV infekcija je dijagnostikovana kod ovaca, koza, svinja i divljih preživara (srne, jeleni, bizoni), kao rezervoari virusa i izvori zaraze u govedima. Posledice BVDV infekcija su abortusi kod gravidnih životinja, loše začeće ženki, mumifikacija i urođene malformacije fetusa, respiratorni problemi, transplacentalne infekcije i fetalna smrt, neonatalni i postnatalni mortalitet, bolesti sluzokože, usporen rast i lošiji rad preživelih životinja. Hemoragijski sindrom (sa trombocitopenijom i krvarenjem) izaziva isključivo necitopatogeni (NCP) BVDV genotip 2, odnosno virulentni sojevi. Osnova programa kontrole je prevencija intrauterine infekcije identifikacijom i uklanjanjem PI životinja iz stada goveda. Velika rasprostranjenost BVDV kod goveda širom sveta i u Srbiji predstavlja opasnost i izaziva izuzetno velike ekonomske gubitke, sprečavajući međunarodnu trgovinu priplodnim i tovnim govedima sa zemljama EU, pre svega zbog neujednačenih pristupa ili nedostatka

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programa kontrole. Postoje sugestije da bi se kontrola bolesti u Srbiji mogla zasnivati na dobrovoljnom programu iskorenjivanja BVDV na nivou stada, zasnovanom na četiri faze, koji podrazumeva česta dijagnostička ispitivanja, uklanjanje PI životinja iz stada i uvođenje strogih mera biobezbednosti. Certifikacija i registar stada sa statusom BVDV slobodnog može se obaviti preko Uprave za veterinu.

Ključne reči: goveda, bovine viral diarrhoea, Pestivirus, BVDV, Prevalencija, Disease kontrola bolesti, ekonomski značaj.

INTRODUCTION

Significant economic losses as a consequence of BVDV infections in cattle have been proven and calculated worldwide (1-3). BVDV are a diverse group of positive-sense single-stranded RNA viruses belonging to the genus Pestivirus within the family Flaviviridae (4). BVDV is classified into 2 types or genotypes: BVDV-1 and BVDV-2, and these two genotypes are divided into several subgenotypes BVDV-1a-1k and BVDV-2a-2c2nd (5-8). Recently, phylogenetic analysis identified bovine pestiviruses and classified them into subgenotypes: at least 21 BVDV-1 (1a–1u), four BVDV-2 and four HoBi-like subgroups (9,10). The latest version of the Flaviviridae taxonomy was ratified in 2023, in which the species names were changed in a conciliatory manner: Pestivirus bovis (from Pestivirus A), Pestivirus tauri (from Pestivirus B) and Pestivirus braziliense (from Pestivirus H) (11).

In addition to the division of genotypes, there are also biotypes based on the presence or absence of a visible cytopathogenic (CPE) effect in infected cell cultures: cytopathogenic (CP) or non-cytopathogenic (NCP) BVDV (12). BVDV is present in cattle on all continents and subcontinents, except for small isolated, exotic areas such as Madagascar. The frequency of infection varies from country to country and from herd to herd, and causes high morbidity and mortality resulting in high losses in the cattle industry (13). Cattle infection caused by a pestivirus known as BVDV, or mucosal disease (MD), is characterized by the following clinical symptoms and changes: erosive inflammation of the mucosa of the digestive tract, diarrhea, mucopurulent nasal discharge, fever, and cachexia. Younger animals from 3 to 8 months are especially susceptible. In addition to acute infection, the infection can also be in a chronic form, and often occurs unintentionally, without clinical symptoms and is persistent in nature, accompanied by immunosuppression and immune tolerance. Abortions and poor conception of females, mummification and congenital malformations of fetuses seriously compromise the reproductive performance of breeding cattle in herds (14).

BVDV infections cause respiratory and reproductive problems: transplacental infection and fetal death, neonatal and postnatal mortality, mucosal disease, stunted growth and poor performance in surviving animals (15,16). BVD is most often manifested clinically after 5 to 7 days of incubation and in an acute form, when morbidity is high and mortality in young individuals is up to 90%, while it is extremely low in the elderly. It most often occurs as an infection in a subclinical course. The most frequently affected animals are 6 to 24 months old. MD is a sporadic form of BVD infection in cattle with clear clinical signs and almost always fatal outcome, acute or chronic course. Morbidity is low and mortality extremely high. The disease usually occurs in cattle 6-24 months of age, and disease onset is associated with immune tolerance in cattle persistently infected (PI) with BVDV.

Major economic losses as a result of BVDV infection include the occurrence of PI calves, which can develop fatal "mucosal disease" (17-19). Placental infection with an NCP virus strain in the first trimester of gestation can induce persistently viremic calves (19), while fetal infection later in gestation often causes abortion, delayed development, or healthy virus-free and seropositive offspring (20,18). PI lifelong carriers of the virus play a key role in the epidemiology of BVDV.

Hemorrhagic syndrome (with the occurrence of thrombocytopenia and hemorrhage) is caused exclusively by NCP BVDV genotype 2, high virulent strains. They affect all age categories of cattle. Cho et al., (21) describe the occurrence of severe hemorrhagic diarrhea in two one-year-old Korean cows in the same herd. The first infected cattle had severe dehydration and died after 3 days. Another beef had anorexia, depression and severe diarrhea with mucus and blood and was necropsied. BVDV2a was detected in the tissues of his digestive tract. Extensive lesions (erosions, ulcerations and extensive bleeding) were observed on the mucous

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membrane of the digestive tract. Immunohistochemically, an extremely positive staining for BVDV2a antigen in the large intestine was diagnosed, which all indicated a hemorrhagic disease caused by BVDV2a in an autochthonous Korean animal. BVDV is one of the most enigmatic and problematic viral agents that lead to BRDC in coinfection. BVDV causes infections in domestic and wild ruminants worldwide (4). In addition to cattle infection, BVDV also infects sheep, goats, pigs and wild ruminants (roe deer, deer, bison), which can be reservoirs of the virus in nature, and thus the source of cattle infection (22,23). Cross-infection between cattle and sheep infected with BVDV and BDV has been demonstrated (24-27). Syndrome X, described in sheep in France, has a multifactorial etiology, and BDV and BVDV antibodies were detected by serological methods. In some cases, other pestiviruses were also isolated. Hyena disease of cattle was first reported in France and recognized in many other countries and was described as a skeletal disorder, and BVDV was identified as the cause of this disease (28,29). To date, little progress has been made in understanding the potential transmission of BVDV from cattle or other ruminants to humans.

Two clinical syndromes were previously described in which pestivirus infections are associated with microencephaly in children and diarrhea of noogenic etiology. Low titers of neutralizing antibodies (Ab) to ruminant pestiviruses were detected in dams that gave birth to micro encephalic calves (30). Feces of children suffering from diarrhea of unknown etiology were found to contain BVDV antigen (17).

There is an urgent need to create fast and accurate diagnostic methods for the detection and control of cattle diseases, and today existing methods are significantly improved or new methods are designed. One of the latest and very interesting methods is recombinase polymerase amplification (RPA). This is a nucleic acid level technique different from PCR, which predominantly involves 3 enzymes, namely, binding single-stranded nucleic acid recombinase (T32 UvsX), single-stranded DNA-binding protein (SSB), and strand-replacing DNA polymerase (Bsu polymerase) (31). An interesting development is observed in the study of Jiang et al. (32), an RPA assay was established for the rapid, sensitive and simultaneous detection of BVDV and BoHV-1 without the need for expensive laboratory equipment, which to the best of the authors' knowledge is the first report on the simultaneous detection of BVDV and BoHV-1 using RPA. The advantages over other known detection techniques are time-saving (completed in less than 30 minutes), does not require thermal cycling and is suitable for non-instrumented platforms for nucleic acid amplification. RPA represents a rapid and efficient method for the simultaneous detection of BVDV and BoHV-1, especially for laboratories with limited resources.

PREVALENCE OF BVD INFECTION AMONG CATTLE WORLDWIDE WITH SPECIAL REFERENCE TO THE ACTUALLY STATUS IN SERBIA

Pioneering research on the representation of BVDV in Serbia was carried out by Belić et al. (97), by examining 224 samples of bovine serum from 6 farms located in Banat and the surroundings of Belgrade and Valjevo for the presence of antibodies to neutralize the BVD virus (VN). A positive titer was determined in 74% of samples (1:9 to 1:2187). The percentage of seropositive animals on certain farms was in the range of 38.8% (91%). The high difference in seroprevalence in different farms was related to the housing system and the age of the animals.

Kurćubić (33) conducted research on dairy farms where the percentage of BVDV seropositive animals varied between 30.55 and 52.24%, depending on age category and herd management practices. On another farm with fattening cattle (aged 6 to 7 months), 55.81% of seropositive sera were detected. The first extensive research that detected antibodies against BVDV in the sera of cattle in the Južno-Bački and Srem districts with the VN test was conducted in 1999-2000 (34).

Serum samples were taken from cattle older than 6 months. VN antibodies against the C24V strain of BVDV were tested in 2546 sera and 46.50% of the samples were positive. The presence of VN antibodies against the NADL strain was examined in 2657 sera, with 50.96% of positive samples. The presence of antibodies against AD-8 strain in 2657 sera was positive in 50.77% of samples.

Petrović et al. (35) detected ncp BVDV isolates 0016 and 0017 and cp isolate Belgrade using indirect immunostaining technique. The OIE reference laboratory (Agency for Veterinary Laboratories, Weybridge, Great Britain - letter dated December 9, 2002) confirmed the official registration of BVDV infection in cattle in Serbia and Montenegro. Isolates 0016 and Belgrade were genetic subtype BVDV 1f. Isolate 0017 belongs to subtype 1b, which was proven by direct sequencing of PCR products and phylogenetic analysis. Serological examinations of 12,083 blood samples of cattle older than 6 months in Serbia revealed 4,647 (38.46%) positively tested animals (36). Kurćubić et al. (37) were the first to attempt to identify PI cattle with BVDV among dairy cattle that were seronegative in both paired sera assays (day 1 and 28), using serum BVDV antigen (Ag) ELISA testing, but in both assays BVDV Ag not confirmed.

Kurćubić et al. (38) tried to identify the presence of PI in cattle of different ages from herds with different production purposes (fattening/milking). BVDV antigen was not detected even in the tested dairy and fattening cattle. The explanation for the impossibility of detecting PI cattle lies in the fact that the untested serums of all animals from farms whose age permitted this test, as well as the known fact that PI cattle are represented in herds in a very small percentage (0.75-2%).

Kurćubić et al. (39) examined blood sera collected from sheep of different age categories reared in different systems for the presence of BVDV and border disease virus (BDV) infections by VNT. It was an attempt to clarify the possibility of transmission to livestock and vice versa. Samples obtained from sheep are grown on five mini-farms where there was no cohabitation of small and large ruminants in the same facilities and 5 sheep farms where cattle are also raised. Blood samples were taken from 5 young sheep (up to 12 months) and 5 from older sheep per farm. Serological testing did not detect the presence of anti-BVDV specific Ab for both BVDV genotypes in any of the 100 blood serum samples tested, such as anti BDV Ab to the Moredun strain of BDV.

As part of their doctoral dissertation, the immunogenicity of two experimentally prepared inactivated vaccines (mono and polyvalent) containing BVDV reference and field strains was evaluated by Kurćubić et al. (40). Tested fattening calves were divided into 3 experimental groups: 10 calves vaccinated twice (1^{st} and 28^{th} day) subcutaneously (s.c.) with 2 mL of inactivated polyvalent vaccine per animal (I group); 10 calves vaccinated in the same way with inactivated monovalent vaccine (Group II) and 9 unvaccinated calves constituted the control group (Group C). Blood sera were collected from immunized animals by a standard procedure: 0, 14, 28, 42 and 56 days after immunization, and tested by VN test. The immune response developed faster and the geometric mean titer (GMT) values for BVDV specific neutralizing Ab were significantly higher in samples from calves from Group II.

Debeljak et al., (41) in one study confirmed with a comprehensive clinical and pathohistological examination and laboratory molecular analyzes the occurrence of mucous membrane disease in one cattle in the field. The animal is clinically suspected of having mucosal disease during routine epidemiological and clinical observation in the field. Isolation of the virus in the MDBK cell line showed a cytopathogenic (CP) effect after 48 hours from samples of transformed parts of the tongue, gums and dental pad. RT-PCR revealed that the isolated virus belonged to BVDV1a genotype and CP biotype. This was confirmed by comparing the 5' UTR sequence with the sequence of viral representatives of some genotypes of BVDV and other pestiviruses (from NCBI GeneBank), as well as the results of virus isolation in a cell line. The stated facts and realistic predictions of the potential and possibility of spreading BVDV in Serbia indicate the undoubted need to introduce a program to control the infection of cattle with BVDV.

İnce and Sait (42) conducted research over a period of two years (from March 2017 to April 2019) to determine the prevalence of BVDV and detected the virus in cattle in dairy farms in the Central Anatolia region (Türkiye), using molecular methods. In this cross-sectional study, a total of 393 blood serum samples from 24 farms were collected by random sampling. The presence of antibodies was tested by a virus neutralization assay using NADL, the reference strain of BVDV. The samples were checked for the presence of BVDV-specific antibodies (Ab) and titer values with the serum neutralization test. Serum samples were tested for the presence of BVDV-specific antigens and specific RNA with a commercial ELISA kit and RT-PCR method, respectively. Seropositivity at the level of animals and herds was at the level of 55.72% (219/393) and 79.16% (19/24), respectively. Seropositivity was statistically significantly different between age groups (χ^2:11.81; p=0.002): prevalence of 45.13%, 60.53% and 73.07% of seropositive in the age groups of 6 months-2 years, 2-5 years and above 5 years. All tested samples for detection of persistently infected animals were negative for antigen and BVDV-specific RNA. The presence of

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BVDV infection in dairy cattle enterprises in the province of Konya has been proven, so it is recommended that it is necessary for the country's economy to prevent the spread of the infection and implement voluntary eradication programs.

The study of Werid et al. (43) focused to investigate the prevalence and risk factors associated with BVDV infections in cattle populations through a systematic review and meta-analysis. The health condition of the examined cattle significantly influenced the prevalence of BVDV. Cattle with bovine respiratory disease complex (BRDC) showed higher levels of antibodies (prevalence of 0.67) and antigens (prevalence of 0.23) compared to cattle with reproductive problems (prevalence of 0.13) or diarrhea (prevalence of 0.01). Cattle age had an effect on prevalence (higher rates in adult cattle compared to calves). Breeding and reproductive risk factors are associated with increased prevalence. Coinfections with pathogens such as bovine herpesvirus-1 or *Neospora caninum* are positively correlated with a higher prevalence of BVDV. Poor management practices such as mixing, introduction of new cattle and direct contact with neighboring farms affect the spread. Larger herds and the presence of PI cattle are associated with higher prevalence. The study indicated the importance of detection methods and risk factors in epidemiological BVDV studies.

BVDV-1a strain was detected as the main subtype in cattle imported from Australia to West Java, Indonesia. BVDV infection includes 12 characteristic symptoms synergized with a positive pattern in PCA, all of which may facilitate the development of preventive/control measures to limit the circulation of BVDV in Indonesia (44).

Birhanu et al. (45) conducted a cross-sectional study of 337 blood serum samples originating from 17 randomly selected farms (out of 133 registered dairy farms in Ethiopia), with the aim of detecting seropositive cattle and BVDV antigen in herds without a history of BVDV vaccination. Antibodies against BVDV were detected by indirect enzyme-linked immunosorbent assay (I-ELISA). In contrast, an antigen capture ELISA has been used to detect BVDV antigen in seronegative animals. The total seroprevalence at the animal level was 15.4%, and 64.7% of herds had at least one seropositive animal. Out of 285 seronegative animals, one animal (0.4%) was positive for BVDV antigen. The same animal was found to be positive by double checking after 21 days. In this study, cows with a history of abortion (OR = 6.3; 95% CI: 1.61 -13.1), a history of repeated breeding (OR = 7; 95% CI: 2.5 - 14.3), animals intensive care (OR = 4.6; 95% CI: 1.6 - 13.0) and multiparous cows (OR = 3.6; 95% CI: 1.5 - 8.9) had a higher proportion of sero-reactors in its comparison category ($p<0.05$). Cows that gave birth to calves with congenital defects (OR = 15.2; 95% CI: 3.2 - 73.6), adult head age groups (OR = 2.9; 95% CI: 1.0-7.9) and cows raised by both artificial and natural mating (OR = 4.6; 95% CI: 1.7 - 12.6) were statistically associated with BVDV seropositivity ($p<0.05$). Therefore, given the proven presence of PI individuals in stadiums in Ethiopia, an urgent control intervention involving screening, culling of PI animals and vaccination is warranted.

In analyses of Dunowska et al. (46), the New Zealand 5′ UTR sequences were closely related to each other, although they did not form one well-supported cluster in a phylogenetic tree and BVDV from New Zealand belong predominantly to the BVDV-1a genotype. The majority were most closely related to international BVDV-1a sequences, with the others few clustering with international BVDV-1c sequences.

Of the BVDV genotypes proven to date, the highest prevalence in China cattle is recorded by BVDV1 (47).

Nugroho et al. (48) reported that the BVDV is endemic in 10 Indonesian provinces, with recognized differences in prevalence between regions. The predominant BVDV subgenotypes circulating in cattle herds in Indonesia are BVDV-1a and BVDV-1c. The most likely sources of BVDV transmission in Indonesia are live cattle imported from Australia or contaminated semen from diseased bulls used in artificial insemination. The impact of this pathogen is most manifest on the reproductive performance of cows. Central government needs to facilitate national regulation, surveillance and incentives to implement strategies to control BVDV infections in different sectors within the Indonesian livestock industry.

PROGRAMS FOR THE CONTROL OF BVDV INFECTIONS

The main purpose of implementing control programs is to prevent intrauterine infection by identifying and removing PI animals from cattle herds. Two different types of BVDV infection control are described in the literature, where the first type involves the identification and removal of PI

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animals and all known sources of infection, as well as the frequent implementation of diagnostic control procedures, which should identify and indicate herds absolutely free from BVDV infection. Strict biosecurity measures are applied to prevent PI animals from entering the herd by testing cattle for BVDV. Another type of control involves vaccinations (with different types of vaccines) to ensure an adequate level of immunity in the herd and bull expedient application of milder control measures (49-51). All programs have advantages and disadvantages, but the precise costs and economic impact of each created strategy must be determined, using different models and methods (52-55).

Certain EU countries have implemented control strategies for BVDV, with a long-term aim of eradicating the virus from national herds (56). In the Scandinavian countries, Austria and Switzerland, programs to control BVDV infection in cattle without vaccination are in force. Another type of control program includes vaccination as an optional and additional means of control and is implemented in Germany, Belgium, Ireland and Scotland. A third type of control program to control BVDV infections in cattle is combined programs (57).

Barrett et al. (58) revealed the potential benefits the eradication of BVD will bring to the control of BoHV-1. In Europe, there are two basic types of systematic programs for the control of BVDV infections, those that allow and those that prohibit vaccination. Belgium, Germany, Ireland and Scotland have a similar approach: removal of PI individuals and optional vaccination. The ultimate goal of vaccination is to prevent fetal infection and the emergence of new PI calves. Most countries with systematic BVD control programs without vaccination have reported serious economic damage from the reintroduction of BVDV into cattle populations that have become seronegative and vulnerable after the removal of PIs as "natural vaccinators". Strict biosecurity measures are necessary to prevent re-introduction of the infection into the fields, along with education and continuous motivation of farmers (59).

A BVDV infection control and eradication program was pioneered in the Scandinavian countries in the early 1990s, which included the application of cost-effective diagnostic methods. In general, vaccination was never an option for their decision makers. Considering the fact that vaccination against BVDV was never applied in those countries, it was possible to apply serological methods to identify herds with active BVD infection. The development of ELISA techniques for the detection of BVDV antigens and virus-specific antibodies has enabled cost-effective, low-cost mass screening (60).

Norway was the first country to eradicate this disease in 1993. Combined milk samples from 26,430 samples were examined by indirect ELISA test. Other Scandinavian countries followed suit. The control program was based on: 1) identification of the free herd 2) prevention of infection in this herd and 3) reduction of the number of infected herds. The reduction in the number of infected herds was achieved by identifying and removing PI individuals from the herd, as well as by preventing the possibility of acute infection (61). In Sweden, the control program started in 1993 (62). The Danish program was designed on the same principles and was launched in 1994 (61,63). At the very beginning of the control program, Finland had a huge advantage in a very small number of herds (only a few) positive for the presence of BVDV, and the control program was implemented in the period from 1998 to 2004 (64).

A voluntary BVDV control program called “BVDV-free” has been implemented in the Netherlands since 1997 and surveys in dairy herds were conducted in the interval from August 1, 2007 to August 1, 2013 (65). The program is designed to be implemented at the herd level with a “test and culling” approach, after which BVDV status is monitored by testing young stock for BVDV antibodies or antigen testing of newborn calves. A serious challenge in implementing the program was the lack of legal frameworks or subsidies, so it was hard to motivate farmers to pay all the costs. The only clear benefit for farmers was the eradication of BVDV from their farms. During the above study period, the percentage of dairy farms in the Netherlands with a "BVDV-free" status increased from 13% to 24%, and the prevalence of active BVDV infections in Dutch dairy herds decreased, which may be correlated with the increasing number of participants in the program "BVDV-free".

Yu et al. (66) investigated the economic (gross margins) and production effects (milk yield, somatic cell count and calving interval) in herds certified "BVDV-free" from Dutch dairy herds between 2014 and 2019, in the final stages of the Dutch national program "without BVDV". This study was designed as a case-control study: the case herds in the first

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analysis are those whose BVDV status changed from "BVDV free" to "BVDV free" during the study period. In another analysis, herds started participating in the Dutch "BVDV-free" program during the study period and received a "BVDV-free" certificate. Control herds in both analyzes were "BVDV free" throughout the study period. The results show that there was no significant change in milk yield, somatic cell count, calving interval and gross margin after "BVDV-free" certification. In this study, the effects of "BVDV-free" certification may have been underestimated, given that the Dutch BVDV control program became mandatory during the study period, and some of the case herds may never have experienced any BVDV infection.

The results suggest that in the final phase of the BVDV control program, the program may no longer have a clear benefit for the herd performance of the participating dairy herds. When designing national programs for the eradication of BVDV, it is necessary to include incentives to motivate the owners of such farms to join the program.

Based on the satisfactory results of the control program in the Scandinavian countries, Austria started implementing a very similar program in 2004 (67) and Switzerland started implementing the program in 2008. The strategy differed from that shown for the Scandinavian countries, due to the high prevalence of BVDV (> 80% of seropositive cattle). During the first year of program implementation, all cattle at the national level were virologically tested and proven PI cattle were removed. From 2009-2012, all newborn calves were analyzed for BVDV earwax samples. The applied strategy and specific measures reduced the prevalence of PI from 1.3% to 0.02% (68). Serological monitoring has been carried out since 2013. Dairy herds are monitored with mass milk samples. Animals in fattening herds are monitored by serological blood testing.

Vaccination against BVD is prohibited in the Scandinavian countries, Austria and Switzerland.

The main risk factors for the spread of BVD virus infection are the sale and other movement of animals between herds, live attenuated/modified vaccines (MLV), semen and embryos. Prevention of the import of infected sperm is achieved by regular serological examination of seronegative bulls and/or examination of the sperm of all seropositive bulls (69). Regulations for artificial insemination centers in the EU introduced strict control of bulls against BVD infection (61). Embryos are dangerous if they are not properly washed and the donor is either a PI or an acutely infected animal. Attenuated vaccines are also a constant threat due to frequent contamination with BVD viruses of both genotypes (69). One of the major factors hindering the development of universally effective BVDV vaccines is their genomic diversity (70).

All the mentioned programs implemented in the Scandinavian countries have provided affirmative results. After several years of program implementation, most of these countries were in "BVDV-free" status. Subsequent analyzes revealed that the implementation of the program was cost-effective (71). The control program without the use of vaccination has a weak point in the final phase, after the removal of PI animals, when the spread of BVDV in the herd is reduced and the remaining animals are seronegative. Reintroduction of BVDV into susceptible herds results in maximum damage in terms of acute clinical disease and effects on fertility.

In the Netherlands, a voluntary BVDV control program has been established in 1997 (cattle herds can obtain a BVDV-free status after a full herd screening for BVD virus). Since 2018, the control program became mandatory for dairy herds. The BVDV national herd-level prevalence in dairy herds in the Netherlands declined from 26% (2004) to 8.7% (2016) and the number of BVDV-free and BVDV-unsuspected herds increased. The risk of (re)introduction of BVDV through the purchase of cattle in herds participating in the national Dutch BVDV-free program is limited. However, the (re)introduction of viruses can have a large impact and result in large economic losses for an individual herd. In a country or region that has a successful BVDV control program, the prevalence of BVDV will decrease, leading to an increased proportion of susceptible cattle in the population. In such a situation, the impact of a new outbreak increases, and thus the importance of controlling the risk of purchase. Therefore, to support BVDV eradication in the Netherlands, it remains important to limit the spread of new BVDV infections through cattle introductions. Testing purchased cattle for virus and antibodies was probably helpful in preventing the spread of infection. This conclusion may also apply to other countries where there is a BVDV control program (72).

Very interesting and practical results were reported in their study carried out in Australia by McMorrow et al. (73). The responsibility and costs

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of controlling and preventing BVDV infections are solely borne by the manufacturer. Veterinarians support farmers and impart knowledge on farm-specific BVDV management practices and facilitate regional BVDV control and elimination. Veterinarians were surveyed to determine their knowledge, attitudes and practices (KAP) related to BVDV control in South East Australia. It was discovered that the recommendations of veterinarians are not always in line with the control and biosecurity measures implemented by farmers. Veterinarians were uncertain about the prevalence of BVDV and the percentage of producers implementing BVDV control measures in the regions where they provide support and health care. Veterinarians generally promoted biosecurity and vaccination, and were concerned about the welfare and additional disease risks associated with persistently infected (PI) cattle. Veterinarians have raised concerns about the risk of disease associated with lack of traceability (a previously undocumented practice whereby producers collected blood from PI cattle to administer to cattle that did not test positive for BVDV, termed “vampire vaccination” in this study). A deeper understanding of the burden, impact and economics of BVDV is needed to align veterinarian and producer KAP and improve on-farm management of BVDV. More mutual appreciation of the values of veterinarians and producers is needed before BVDV control can be implemented at a regional or national level.

Strict application of biosecurity measures must be the basis of any control plan to eradicate BVDV. Cattle vaccination as the basis of a control program is interesting because it is a cheap and relatively effective method for preventing infections with the virus that causes BVD. The most important goals of cattle vaccination are protection of the fetus, prevention of clinical diseases and immunosuppression. The most widely used is the modified live vaccine (MLV) against BVD, monovalent or polyvalent - in combination with other viral or bacterial agents (74). Most modern MLVs are created from cp BVDV, because cp viruses are not risky and do not lead to PI fetuses. They induce strong humoral and cellular immunity and provide solid fetal protection. In the EU, the use of MLV in unvaccinated animals during the first 6 months of pregnancy is not recommended. 7-week-old calves can be vaccinated against BVD because after vaccination they develop a T cell-mediated immune response despite circulating maternal antibodies (75).

Safety concerns have prompted the development of inactivated-killed vaccines (KV), which could be administered at any age and period of pregnancy (76). KVs have a disadvantage compared to MLVs because they must be given multiple times to achieve protection. Immunity after KV administration occurs after at least 3 to 4 weeks, while MLVs provide protection within a few days. Fetal protection varies from incomplete to satisfactory (77,76). The immune response to KV has recently been enhanced by the addition of potent adjuvants. Humoral immunity after KV administration is usually strong, and cellular immunity varies from incomplete to strong (78,79). Killed BVDV vaccines (KV) are predominantly present on the EU market. Another disadvantage of KV is the necessary annual revaccination. Vaccine administration has not changed BVD prevalence over time (80), due to unique biology of BVDV infections, and ability to produce PI progeny creates a virus reservoir that is continuously shedding large amounts of infectious virus. Thus, PI cattle have ample opportunity during their lifetime to meet and infect new susceptible animals, even when the general level of immunity against BVD is high. This is in contrast to most other infectious diseases where shedding is temporally limited to a few days or weeks. If PI animals are present in the herd, only a herd immunity of 100% will prevent the emergence of new PI calves, which is impossible to achieve under practical conditions. The consequence of the aforementioned facts is that so far there is no data globally on the successfully implemented control strategy for the control of BVDV bovine infections, which is based exclusively on the implementation of vaccination.

Combined control programs were implemented in Germany between 2004 and 2011, in regions with a dense livestock population (about 200 animals per km^2) and intensive trade, and involve a combined approach of testing and culling PI animals plus optional vaccination. The local epidemiological situation dictates the measures that will be prescribed by the veterinary authorities, i.e. prohibition or recommendation of vaccination.

The implementation of combined programs gave good results: the prevalence of PI cattle decreased from 0.48% in 2011 to 0.02% in 2016 (98). Strategies for the systematic eradication of BVDV infection have been launched in a number of

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European countries, created by the government and the relevant authorities or farmers' associations with the support of the government. They are similar to the above, with certain minor modifications. In 2019, the average farm in Slovenia had 15.8 animals. Of the total cattle population, 29.9% is represented by the Simmental breed, 16.8% by Holstein, 4.4% by brown and 0.9% by the Slovenian autochthonous Cika breed. The rest (48.0%) are either crossbred cattle or those of unknown pedigree or fattening breed of cattle (mainly Limousin, Charolais or Angus). The cattle population consists of cows (34.0%), calves (29.8%), heifers (20.8%) and bulls (15.4%) (81).

In Slovenia, a BVDV-free voluntary control program with provisions for recognition, acquisition and maintenance of the status was adopted for the first time. According to the data from the first 7 years of running the program, only a small part of the herd has an officially recognized and maintained BVDV-free status. The program is paid exclusively by farmers, with discounts and efficient work of the Laboratory of the Veterinary Faculty in Ljubljana; the health condition of several herds was improved by the application of the adopted Rulebook. The number of herds with official BVDV-free status is still very small, which nevertheless benefits farmers who sell and buy animals. Also, the voluntary program provides data on the prevalence of BVDV and the estimated number of BVDV positive herds. Austria and Switzerland have already achieved BVDV-free status at the national level. The next step for Slovenia is to move to a mandatory national program to eradicate BVDV and the diseases it causes. However, during the implementation of the first voluntary BVDV control program, several cattle herds achieved significant improvement and progress in health status after implementing preventive measures or successfully maintained BVDV-free status for several years (82).

In an interval of 7 years (2014-2020), a total of 348 herds were included in BVDV antibody testing. A quarter of the examined herds were positive for the presence of BVDV antibodies. In order for the herd to be in "BVDV-free" status, two consecutive ELISA tests at least 6 months apart in all animals aged 7 to 13 months must have negative results for BVDV antibodies (82).

In the same program, 236 herds were involved in the detection of BVDV in individual herds by the RT-PCR method in real time, in order to identify and eliminate positive animals from the herd. At least one animal positive for BVDV was detected in 31.3% of herds, with a total of 267 (2.9%) persistently infected (PI) animals identified. The costs of testing for an average herd, recognized as BVDV-negative, and maintaining the BVDV-free status within the implemented voluntary program, amounted to €97.64/year. For the average positive herd, the laboratory costs for BVDV elimination were €189.59/year and were borne by the farmers themselves, with limited progress towards eradication at the national level (82).

A longer description of the implementation of the BVDV infection control program in Slovenia can be illustrative of the creation of a similar program in Serbia, which is currently missing on national level.

In the Republic of Serbia, the situation is challenging due to the proven high prevalence of BVDV (especially in fattening cattle), different strains of the virus isolated by modern methods and classified on the phylogenetic tree, clinical cases described. The problems have not been comprehensively solved. Control of BVDV infection in cattle herds is sporadic, there are no systemic solutions. This economic and health problem, which seriously affects the competitiveness of Serbian cattle breeding, was recognized by veterinary experts and the leadership of the Veterinary Administration of the Ministry of Agriculture and Environmental Protection, by launching an initiative to establish a voluntary program for the control of BVD infection on the territory of the Republic of Serbia (83) and the development of the working version of the program for the control, suppression and eradication of BVDV infection in the Republic of Serbia was entrusted to the Scientific Institute of Veterinary Science "Novi Sad" (NIV-NS), within the technological development project no. TR 31084. The program was initially intended to be implemented on a voluntary basis on individual farms and cattle herds.

The BVDV infection control program combines successful methodologies developed and applied in several countries (Scandinavia, Germany, Italy and Slovenia), as well as "modern" diagnostic and laboratory methods that will be included in animal testing (84). If it shows good results, the Program would subsequently be implemented at the national level as a state program for the control of BVDV infection in the Republic of Serbia, with the main goal of herds obtaining the status of "free from BVDV" infection. Herd certification would be verified by the Veterinary Administration, which

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would create an online database of certified herds with all relevant data. The program design includes four phases: Phase 1: Confirmation of BVDV-free status, Phase 2: Gain of BVDV-free status, Phase 3: Maintenance of BVDV-free herd status, and Phase 4: Loss of BVDV-free status. and the procedure for restoring and restoring the herd's BVDV-free status.

The objectives of the program are:

• Reduction of economic losses in cattle herds;
• Obtaining certified reproductive material to facilitate and promote its access to the international market and
• Better market price of certified reproductive material.

In light of the facts presented in this review, we also point to an excellent and invaluable contemporary review based on the vast experience of the authors (85) in which the authors state that volunteer programs are particularly difficult to manage because they require a high degree of awareness, motivation and compliance on the part of interested parties, especially participating farmers. Many new programs are the result of discussions with stakeholders. Instead of adopting a simple and rigorous control program based on the positive experiences of other countries, many compromises are often made for ostensibly economic reasons, which actually jeopardize control efforts from the outset. Typical examples are unclear or non-existent regulations regarding the fate of proven PI cattle, weak or no provisions on movement restrictions and biosecurity, and lack of livestock quarantine rules.

If similar shortcomings exist, long program duration, high costs and poor performance, and very often failure, are inevitable. Successful volunteer programs require a strong commitment from all stakeholders and a desire to make the program successful. A steering group of key stakeholders should facilitate communication with farmers and coordinate education and control measures. During the implementation of all phases and activities of the entire program, efforts must be monitored, while respecting the importance of data management. In certain countries, existing livestock databases have been successfully used to support BVD control.

ESTIMATION OF ECONOMIC LOSSES

Cattle diseases caused by BVD infection are the result of transplacental infections that cause fetal death, congenital malformations, neonatal and postnatal mortality, leukopenia and immunosuppression, respiratory syndrome, reduced milk yield, reduced female conception (permanent mortality, periodontal disease), abortions, mummification, immunotolerance and persistent infection (PI), mucosal disease (MD), poor growth and performance of surviving animals (86-89). In southeastern parts of Norway, BVDV infection has been identified as the cause of 15% of all abortions and stillbirths in dairy cows (99).

Calculation of herd-level losses in so-called classic attacks, where many transient infections go unnoticed, and where herd losses are associated with reproductive disorders and PI animals at the level of $25-160 per cow (100). Losses during attacks when BVDV co-existed with other infections or highly virulent strains causing severe disease and high mortality, as well as transiently infected animals, were calculated to exceed $400 per cow (90, 100). Estimates at the national level (in Great Britain, Norway and Denmark) and subsequent recalculation of calving losses suggest that they were on the order of $10-40 per calving (91, 101). Losses when a virulent strain of BVDV is present in the population are estimated at $57 per calf (13).

In the UK, the annual total economic loss from BVD infection is around £120 million. In Denmark, the damage is estimated at 17 million dollars per year (61). BVDV infections are considered the third most economically important disease in livestock, just behind rinderpest and foot-and-mouth disease. The economic consequences of a national eradication program in Norway are expressed as cost-benefit calculations and show that the program was profitable as early as the second year after initiation, and then had a net present value of $6 million over the first 5 years, 1993-1997 (92). The net present value for the entire eradication period was later estimated at approximately $22 million (93). In Denmark, the costs of a medium-term control program were about $3.5 million—low costs compared to the avoided losses—gains of $20 million, calculated before the program began (13).

FUTURE DIRECTIONS

To study BVDV NSP, it is necessary to investigate which host cell genes should be activated during BVDV transcription, as well as which host cell components are necessary for its translation. Some BVDV NSP genes have a role in transcription, translation and regulation of the virus itself, as well as a role and mechanism in host cell antagonism. For the complete prevention of BVDV, it is necessary to strengthen the research of the interaction between BVDV and the host, as well as the interaction between viral structural proteins and non-structural proteins after the entry of BVDV into cells, which is of great importance for the prevention and control of BVD (94).

Workman et al. (95) used CRISPR-mediated homology-directed repair and somatic cell nuclear transfer to generate a live calf with a six amino acid substitution in the BVDV binding domain of bovine CD46. The result was a genetically engineered calf with dramatically reduced susceptibility to infection as measured by reduced clinical signs and a lack of viral infection in white blood cells. The edited calf appears normal and healthy at 20 months of age with no apparent adverse effects of genetic engineering. This precision-bred proof-of-concept animal provides the first evidence that deliberate genomic changes in the CD46 gene can reduce the burden of BVDV-associated disease in cattle and is consistent with our stepwise, *in vitro* and *ex vivo* experiments with cell lines and matched fetal clones.

The consensus panel agrees that continued investment in BVDV awareness and education is important for successful, efficient and effective BVDV control by cattle producers (96).

Identification and prevention risky behaviour within a BVDV control programme and trace routes of BVDV transmission can be performed by a molecular epidemiological approach.

CONCLUSIONS

It has been clearly shown that various BVDV bovine infection control programs and its eradication can be successfully implemented at the national level (Scandinavian countries, Austria, Switzerland, Germany, UK, Australia, New Zealand, Indonesia). Given the described success of the program and visible progress in the control of BVDV infections, in a time interval whose duration varies depending on the initial condition (prevalence of BVDV and percentage of the PI animals). Visible progressions in the control of BVDV infections have been observed. Control programs gave very significant results, and their success was assessed as good usually after a period of implementation of about 5 years. We believe that there are all the conditions to implement the control program as proposed by experts from the Scientific Veterinary Institute „Novi Sad“, which is based on the experiences of European countries and adapted to the specifics of cattle breeding in Serbia. With the support of the Veterinary Administration of Republic of Serbia, the program would grow from a voluntarily phase to a governmental program for the control of BVDV infection.

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REFERENCES

1. *Houe H. Economic impact of BVDV infection in dairies. Biologicals 2003, 31:137-143.* https://doi.org/10.1016/S1045-1056(03)00030-7
2. *Fourichon C., Beaudeau F., Bareille N., Seegers N. Quantification of economic losses consecutive to infection of a dairy herd with bovine viral diarrhoea virus. Preventive Veterinary Medicine 2005, 72:177-181.*
3. *Ridpath J.F. Bovine viral diarrhea virus: global status. Veterinary Clinics of North America: Food Animal Practice 2010, 26:105-121.* https://doi.org/10.1016/j.cvfa.2009.10.007
4. *Lindenbach B.D., Thiel H.J., Rice C.M. Flaviviridae: The Viruses and Their Replication. In: D.M. Knipe and P.M. Howley (Eds.) Fields Virology, 5th edn. Lippincott-Raven Publishers, Philadelphia, 2007.*
5. *Ridpath J.F., Bolin S.R., Dubovi E.J. Segregation of bovine viral diarrhea virus into genotypes, Virology 1994, 205:66-74.* https://doi.org/10.1006/viro.1994.1620
6. *Bachofen C., Stalder H., Braun U., Hilbe M., Ehrensperger F., Peterhans E. Co-existence of genetically and antigenically diverse bovine viral diarrhea viruses in an endemic situation. Veterinary Microbiology 2008, 131:93-102.* https://doi.org/10.1016/j.vetmic.2008.02.023
7. *Strong R., Errington J., Cookl R., Ross-Smith N., Wakeley P., Steinbach F. 2013. Increase phylogenetic diversity of bovine viral diarrhea virus type 1 isolates in England and Wales since 2001. Veterinary Microbiology 2013, 162:315-320.* https://doi.org/10.1155/2014/147145
8. *Luzzago C., Lauzi S., Ebranati E., Giammarioli M., Moreno A., Cannella V., Masoero L., Canelli E., Guercio A., Caruso C., Ciccozzi M., De Mia G.M., Acutis P.L., Zehender G., Peletto S. Extended genetic diversity of bovine viral diarrhea*

virus and frequency of genotypes and subtypes in cattle in Italy between 1995 and 2013. BioMed Research International 2014,147145.
https://doi.org/10.1155/2014/147145

9. *Jenckel M., Hoper D., Schirrmeier H., Reimann I., Goller K.V., Hoffmann B., Beer M. Mixed triple: Allied viruses in unique recent isolates of highly virulent type 2 bovine viral diarrhea virus detected by deep sequencing. J. Virol. 2014, 88:6983–6992. https://doi.org/10.1128/jvi.00620-14*
10. *Yesilbag K., Alpay G., Becher P. Variability and global distribution of subgenotypes of Bovine Viral Diarrhea Virus. Viruses 2017, 9:128. https://doi.org/10.3390/v9060128*
11. *Simmonds P., Becher P., Bukh J., Gould E.A., Meyers G., Monath T., Muerhoff S., Pletnev A., Rico-Hesse R., Smith D.B., Jack T. Stapleton J.T., ICTV Report Consortium 2017. ICTV virus taxonomy profile: Flaviviridae. Journal of General Virology 2017, 98, 2. https://doi.org/10.1099/jgv.0.000672*
12. *Vilcek S, Paton DJ, Durkovic B, Strojny L, Ibata G, Moussa A, Loitsch A, Rossmanith W, Vega S, Scicluna MT, Paifi V. Bovine viral diarrhoea virus genotype 1 can be separated into at least eleven genetic groups. Arch Virol. 2001;146(1):99-115. https://doi.org/10.1007/s007050170194*
13. *Houe H., Pedersen K.M., Meyling A. A computerized spreadsheet model for calculating total annual losses due sensitivity analysis of selected parameters. In Proceedings of 10[th] International Symposium for Veterinary Epidemiology and Economics, 17-21 November 1993, Vina del mar, Chile. International Society for Veterinary Epidemiology and Economics (available at: www.sciquest.org.nz).*
14. *Baker J.C. The clinical manifestations of bovine viral diarrhea infection. Veterinary Clinics of North America: Food Animal Practice 1995, 11:425-445. https://doi.org/10.1016/s0749-0720(15)30460-6*
15. *Bolin S.R., Mc Clurkin A.W., Cutlip R.C., Coria M.F. Severe clinical disease induced in cattle persistently infected with noncytopathogenic bovine viral diarrhea virus. American Journal of Veterinary Research 1985, 46:573-576.*
16. *Roeder P.L., Harkness J.W. BVD virus infection: Prospects for control. Veterinary Record 1986, 118:143-147. http://dx.doi.org/10.1136/vr.119.6.143*
17. *Brownlie J. The pathogenesis of bovine virus diarrhoea virus infections. Review of Scientific Techology 1990, 9:43-59. https://doi.org/10.20506/rst.9.1.491*
18. *Bielefeldt O.H. The pathologies of bovine viral diarrhea virus infection. A window on the pathogenesis. Veterinary Clinics of North America: Food Animal Practice 1995, 11:447-476. https://doi.org/10.1016/S0749-0720(15)30461-8*
19. *Moennig V., Liess B. Pathogenesis of intrauterine infections with bovine viral diarrhea virus. Veterinary Clinics of North America: Food Animal Practice 1995, 11:477-487. https://doi.org/10.1016/S0749-0720(15)30462-X*
20. *Done J.T., Terlecki S., Richardson C., Harkness J., Sands J.J., Patterson D.S., Sweasey D., Shaw I.G., Winkler C.E., Duffell S.J. Bovine virus diarrhea - mucosal disease virus: pathogenicity for the fetal calf following maternal infection. Veterinary Record 1980, 106:473-479.*
http://dx.doi.org/10.1136/vr.106.23.473
21. *Cho H.-C., Kim, B.-S., Jang, D.-H., Lee, K.-H., Kyoung-Seong Choi, K.-S. Hemorrhagic disease caused by bovine viral diarrhea virus-2a in Korean Indigenous Cattle: case reports. Korean Journal of Veterinary Research 2023, 63(1):e7. https://doi.org/10.14405/kjvr.20230005*
22. *Nettleton P.F., Herring J.A., Corrigall W. Isolation of Bovine virus diarrhoea virus from a Scottish red deer. Veterinary Record 1980, 107:425-426. http://dx.doi.org/10.1136/vr.107.18.425*
23. *Cvetnić S. Viral diseases of animals. Yugoslav Academy of Sciences and Arts (JAZU), 1983.*
24. *Osburn B.I., Clarke G.L., Stewart W.C., Sawyer H. Border Disease-like syndrome in lames: antibodies to hog cholera and bovine viral diarrhea viruses. Journal of American Veterinary Medicine Association 1973, 163(10):1165-1167.*
25. *Dahle J., Liess B., Frey H.R. Interspecies transmission of Pestiviruses: experimental infections with bovine viral diarrhea virus in pigs and hog cholera virus in cattle. Proceedings of a seminar in the CEC programme of coordination of research on animal husbandry. Brussels, Belgium, 10-11 September 1985, 195 212.*
26. *Nettleton P.F. Pathogenesis and epidemiology of Border Disease. Annales de Recherches Veterinaires 1986, 18:147-155.*
27. *Brownlie J. The pathways for bovine virus diarrhoea virus biotypes in the pathogenesis of disease. Archives of Virology 1991, Supplementum S3: 79-96. https://doi.org/10.1007/978-3-7091-9153-8_10*
28. *Russo P., Giauffret A., Fleche-Seban C. Occurrence of pestivirus infections in sheep in France. Proceedings of a seminar in the CEC programme of coordination of research on animal husbandry. Brussels, Belgium, 10-11 September 1985, 103-110.*
29. *Nettleton P.F. Pestivirus infections in ruminants other than cattle. Revue Scientifique et Technique De L'Office International Des Epizooties 1990, 9(1):131-150.*
30. *Potts B.A., Sever J.L., Tzan N.A., Huddleston D., Elder G.A. Possible role of Pestiviruses in microencephaly. Lancet 1987, 972-973. https://doi.org/10.1016/S0140-6736(87)90311-4*
31. *Piepenburg, O., Williams, C.H., Stemple, D.L., Armes, N.A. DNA detection using recombination proteins. PLoS Biology 2006, 4: e204. https://doi.org/10.1371/journal.pbio.0040204.*
32. *Jiang L., Zhang G., Wang P., Niu X., Liu Q., Zhang S., Gao W., Li Y. Simultaneous detection of bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BoHV-1) using recombinase polymerase amplification. Scientific Reports 2024, 14:10169. https://doi.org/10.1038/s41598-024-56869-7*

33. *Kurćubić V. Serological examinations of bovine viral diarrhoea virus infection in cattle, Master thesis, Faculty of Veterinary Medicine, University of Belgrade, 1993.*
34. *Petrović T. Estimation of the prevalence of the infection in cattle caused by bovine viral diarrhoea virus. Master thesis, University of Belgrade, 2002.*
35. *Petrović T., Đuričić B., Toplak I., Lazić S., Barlič-Maganja D., Hostnik P., Grom J., Sandvik T. Isolation and confirmation of bovine viral diarrhoea virus in Serbia and comparative typing with recent Slovenian isolates. Acta Veterinaria-Beograd 2004, 54(1):33-42.*
36. *Petrović T. Identification and genetic characterization of BVD virus isolates on the territory of Republic of Serbia. PhD Thesis, Faculty of Veterinary Medicine, University of Belgrade, 2006.*
37. *Kurćubić V., Ilić Z., Djoković R., Jevtić S. Identification of Cattle Persistently Infected (PI) with the bovine viral diarrhea virus (BVDV) using a screening method for a control and eradication Program. Acta Agriculturae Serbica 2009, 14:79-92.*
38. *Kurćubić V., Ilić Z., Djoković R., Jevtić S., Petrović T. Determination of the presence and degree of infection with bovine viral diarrhea virus in cattle herds in central Serbia - possibilities for control. Veterinarski glasnik 2010, 64:3-19. https://doi.org/10.2298/VETGL1002003K*
39. *Kurćubić V., Petrović T., Djoković R., Ilić Z. Investigation of bovine viral diarrhea virus (BVDV) and border disease virus (BDV) infections in different categories of sheep in Southwestern Serbia. Veterinarski glasnik 2010, 64:319-337. https://doi.org/10.2298/VETGL1006319K*
40. *Kurćubić V.S., Milić N., Djoković R.D., Ilić Z.Z. Evaluation of immunogenic properties of monovalent and polyvalent inactivated bovine virus diarrhea virus (BVDV) vaccines, African journal of microbiology research 2011, 5:2422- 2427. https://doi.org/10.5897/AJMR10.892*
41. *Debeljak Z., Milićević V., Vasković N., Tomić A., Petrović N., Bradonjić S. Presentation of appearance of Bovine Viral Diarrhea, Abstracts Book of XI Epizootiology days with International Participation. Apatin, Banja Junaković, 1-4. April 2009, 1-12. ISBN 978-86-83115-11-2.*
42. *İnce Ö.B., Sait A. Molecular and serological investigation of Bovine Viral Diarrhea Virus in dairy cattle in Konya province. Journal of Advances in VetBio Science and Techniques 2022, 7(1):80-88. https://doi.org/10.31797/vetbio.1061489*
43. *Werid G.M., Hemmatzadeh F., Miller D., Reichel M.P., Messele Y.E., Petrovski K. Comparative Analysis of the Prevalence of Bovine Viral Diarrhea Virus in Cattle Populations Based on Detection Methods: A Systematic Review and Meta-Analysis. Pathogens 2023, 12, 1067.https://doi.org/10.3390/pathogens12081067*
44. *Primawidyawan A., Setiyaningsih S., Wulansari R., Subangkit M., Priosoeryanto B.P. Detection and characterization of bovine viral diarrhea virus in beef cattle imported from Australia to West Java, Indonesia. Veterinary World 2023, 16(7):1468–1476. www.doi.org/10.14202/vetworld.2023.1468-1476*
45. *Birhanu W., Tesfaye A., Getachew Y., Negussie H. Seroprevalence of bovine viral diarrhea virus and detection of persistently infected (PI) animals in dairy farms of Holeta, central Ethiopia. Ethiopian Veterinary Journal 2024, 28 (1), 73-87. https://doi.org/10.4314/evj.v28i1.5*
46. *Dunowska M., Lal R., Dissanayake S.D., Bond S.D., Burrows E., Moffat J., Howe L. Bovine viral diarrhoea viruses from New Zealand belong predominantly to the BVDV-1a genotype. New Zealand Veterinary Journal 2024, 72:2, 66-78. https://doi.org/10.1080/00480169.2023.2291039*
47. *Diao N.C. Gong Q.L., Li J.M., Zhao D., Li D., Zhao B., Ge G.Y., Li D.L., Shi K., Du R. Prevalence of bovine viral diarrhea virus (BVDV) in yaks between 1987 and 2019 in mainland China: A systematic review and meta-analysis. Microbial Pathogenesis 2020, 144. https://doi.org/10.1016/j.micpath.2020.104185*
48. *Nugroho W., Silitonga R.J.P., Reichel M.P., Irianingsih S.H., Wicaksono M.S. The epidemiology and control of bovine viral diarrhoea virus in tropical Indonesian cattle. Pathogens 2022, 11(2):215. https://doi.org/10.3390/pathogens11020215*
49. *Harkness J.W. The control of bovine viral diarrhoea virus infection. Annales de Recherches Veterinaires 1987, 18:167-174.*
50. *Ames T.R., Baker J.C. Management practices and vaccination programs that help control BVD virus infection. Veterinary Medicine 1990, 85:1140-1149.*
51. *Brownlie J., Clarke M.C., Howard C.J. Experimental production of fatal mucosal disease in cattle. Veterinary Record 1991, 114:535-536. http://dx.doi.org/10.1136/vr.114.22.535*
52. *Moennig V., Houe H., Lindberg A. BVD control in Europe: current status and perspectives. Animal Health Research Review 2005, 6(1):63-74. https://doi.org/10.1079/AHR2005102*
53. *Heuer C., Healy A., Zerbini C. Economic Effects of Exposure to Bovine Viral Diarrhea Virus on Dairy Herds in New Zealand. Journal of Dairy Science 2007, 90:5428-5438. https://doi.org/10.3168/jds.2007-0258*
54. *Lanyon S.R., Reichel M.P. Understanding the Impact and Control of Bovine Viral Diarrhoea in Cattle Populations. Springer Science Reviews 2013, 1:85-93. https://doi.org/10.1007/s40362-013-0007-6*
55. *Santman-Berends I.M.G.A., Mars M.H., van Duijn L., van Schaik G. Evaluation of the epidemiological and economic consequences of control scenarios for bovine viral diarrhea virus in dairy herds. Journal of Dairy Science 2015, 98:7699-7716. https://doi.org/10.3168/jds.2014-9255*

56. Shortall O. Lessons learned for animal health governance from bovine viral diarrhea eradication schemes in Scotland and Ireland. Frontiers in Veterinary Science 2022, 9, 956635. https://doi.org/10.3389/fvets.2022.956635
57. Moennig V., Becher P. Control of Bovine Viral Diarrhea. Pathogens 2018, 7, 29. https://doi.org/10.3390/pathogens7010029
58. Barrett D., Lane E., Lozano J.M., O'Keeffe K., Byrne A.W. Bovine Herpes Virus Type 1 (BoHV-1) seroprevalence, risk factor and Bovine Viral Diarrhoea (BVD) co-infection analysis from Ireland. Scientific Reports 2024, 14:867. https://doi.org/10.1038/s41598-023-50433-5
59. Metcalfe L.V.A. An Update on the Status of BVD Control and Eradication in Europe. Journal of Veterinary Science & Medicine 2019, 7(1):4.
60. Gottschalk E.E., Greiser-Wilke I., Frey H.R., Liess B., Moennig V. An antigen capture test for the detection of cattle viremic with bovine viral diarrhoea virus - a comparison with BVD virus isolation from buffy coat cells in bovine kidney cells. Zentralbl Veterinarmed B 1992, 39:467-472. https://doi.org/10.1111/j.1439-0450.1992.tb01195.x
61. Bitsch V., Ronsholt L. Control of bovine viral diarrhea virus infection without vaccines. Veterinary Clinics of North America: Food Animal Practice 1995, 11(3):627-640. https://doi.org/10.1016/S0378-1135(00)00270-4
62. Hult L., Lindberg A. Experiences from BVDV control in Sweden. Preventive Veterinary Medicine 2005, 72:143-148. https://doi.org/10.1016/j.prevetmed.2005.04.005
63. Bitsch V., Hansen K.E., Ronsholt L. Experiences from the Danish programme for eradication of bovine virus diarrhoea (BVD) 1994-1998 with special reference to legislation and causes of infection. Veterinary Microbiology 2000, 77:137-143. https://doi.org/10.1016/S0378-1135(00)00270-4
64. Rikula U., Nuotio L., Aaltonen T., Ruoho O. Bovine viral diarrhoea virus control in Finland 1998-2004. Preventive Veterinary Medicine 2005, 72:139-142. https://doi.org/10.1016/j.prevetmed.2005.08.010
65. van Duijn L., Veldhuis A.M.B., Mars M.H., de Roo B., Lam T.J.G.M. Efficacy of a voluntary BVDV control programme: Experiences from the Netherlands, The Veterinary Journal 2019, 245, 55-60, https://doi.org/10.1016/j.tvjl.2018.12.016.
66. Yue X., Wu J., van der Voort M., Steeneveld W., Hogeveen H. Estimating the Effect of a Bovine Viral Diarrhea Virus Control Program: An Empirical Study on the Performance of Dutch Dairy Herds. Frontiers in Veterinary Science 2022, 9:892928. https://doi.org/10.3389/fvets.2022.892928
67. Schoepf K., Revilla-Fernandez S., Steinrigl A., Fuchs R., Sailer A., Weikel J., Schmoll F. Retrospective epidemiological evaluation of molecular and animal husbandry data within the bovine viral diarrhoea virus (BVDV) control programme in Western Austria during 2009-2014. Berliner und Münchener tierärztliche Wochenschrift 2016, 129:196-201.
68. Bachofen C., Stalder H., Vogt H.R., Wegmuller M., Schweizer M., Zanoni R., Peterhans E. Bovine viral diarrhea (BVD): From biology to control. Berliner und Münchener tierärztliche Wochenschrift 2013, 126:452-461. https://doi.org/10.2376/0005-9366-126-452
69. Lindberg A.L., Alenius S. Principles for eradication of bovine viral diarrhoea virus infections in cattle populations. Veterinary Microbiology 1999, 64:197- 222. https://doi.org/10.1016/S0378-1135(98)00270-3
70. Falkenberg S.M., Dassanayake R.P., Terhaar B., Ridpath J.F., Neill J.D., Roth J.A. Evaluation of antigenic comparisons among BVDV isolates as it relates to humoral and cell mediated responses. Frontiers in Veterinary Science 2021, 8, 685114. https://doi.org/10.3389/fvets.2021.685114
71. Valle P.S., Skjerve E., Martin S.W., Larssen R.B., Osteras O., Nyberg O. Ten years of bovine virus diarrhoea virus (BVDV) control in Norway: A cost-benefit analysis, Preventive Veterinary Medicine 2005, 72: 189-207. https://doi.org/10.1016/j.prevetmed.2005.07.017
72. Van Duijn L., Santman-Berends I., Biesheuvel M., Mars J., Waldeck F., van Schaik G. Why Test Purchased Cattle in BVDV Control Programs? Frontiers in Veterinary Science 2021, 8:686257. https://doi.org/10.3389/fvets.2021.686257
73. McMorrow C., Gunn A.J., Khalfan S., Hernandez-Jover M., Brookes V.J. Veterinarians' Knowledge, Attitudes and Practices Associated with Bovine Viral Diarrhoea Virus Control and Prevention in South-East Australia. Animals (Basel) 2020, 11, 10(9):1630. https://doi.org/10.3390/ani10091630
74. Van Oirschot J.T., Bruschke C.J.M., van Rijn P.A. Vaccination of cattle against bovine viral diarrhoea, Veterinary Microbiology 1999, 64:169-183. https://doi.org/10.1016/S0378-1135(98)00268-5
75. Endsley J.J., Roth J.A., Ridpath J., Neill J. Maternal antibody blocks humoral but not t cell responses to BVDV. Biologicals 2003, 31:123-125. https://doi.org/10.1016/S1045-1056(03)00027-7
76. Newcomer B.W., Walz P.H., Givens M.D., Wilson A.E. Efficacy of bovine viral diarrhea virus vaccination to prevent reproductive disease: A meta-analysis. Theriogenology 2015, 83:360-365. https://doi.org/10.1016/j.theriogenology.2014.09.028
77. Rodning S.P., Marley M.S., Zhang Y., Eason A.B., Nunley C.L., Walz P.H., Riddell K.P., Galik P.K., Brodersen B.W., Givens M.D. Comparison of three commercial vaccines for preventing persistent infection with Bovine Viral Diarrhea Virus. Theriogenology 2010, 73:1154-1163. https://doi.org/10.1016/j.theriogenology.2010.01.017

78. *Platt R., Coutu C., Meinert T., Roth J.A. Humoral and t cell-mediated immune responses to bivalent killed ovine viral diarrhea virus vaccine in beef cattle. Veterinary Immunology and Immunopathology 2008, 122:8-15. https://doi.org/10.1016/j.vetimm.2007.11.009*
79. *Stevens E.T., Zimmerman A.D., Butterbaugh R.E., Barling K., Scholz D., Rhoades J., Chase C.C. The induction of a cell-mediated immune response to bovine viral diarrhea virus with an adjuvanted inactivated vaccine. Veterinary Therapeutics: Research in Applied Veterinary Medicine 2009, 10, E1-8.*
80. *O'Rourke K. BVDV: 40 years of effort and the disease still has a firm hold. Journal of the American Veterinary Medical Association 2002, 220:1770-1773. http://dx.doi.org/10.1590/S0100-736X2009000100006*
81. *Sadar M., Jeretina J., Logar B., Opara A., Perpar T., Podgoršek P. 2020. Results of Dairy and Beef Recording. Sadar Ljubljana M, editor. Kmetijski inštitut Slovenije: Slovenia (2020). Available online at: http://www.govedo.si.*
82. *Toplak I., Hostnik P., Cerne D., Mrkun J., Starič J. The Principles of the Voluntary Programme for the Control and Elimination of Bovine Viral Diarrhoea Virus (BVDV) From Infected Herds in Slovenia. Frontiers in Veterinary Science 2021, 8:676473. https://doi.org/10.3389/fvets.2021.676473*
83. *Petrović T., Lazić S., Bugarski D., Lupulović D., Polaček V., Vidanović D., Plavšić B. The proposal of a program for control, suppression and eradication of Bovine Viral Diarrhea Virus infection in cattle herds in the Republic of Serbia. Arhiv veterinarske medicine 2015, 8(2):35-53.*
84. *Petrović T., Velhner M., Petrović J., Stojanov I., Grgić Ž., Lazić, S. Modern laboratory diagnostic methods in veterinary medicine and the possibility of its application. Arhiv veterinarske medicine 2010, 3(1):39-61.*
85. *Moennig V., Yarnall M.J. The Long Journey to BVD Eradication. Pathogens 2021, 10:1292. https://doi.org/10.3390/pathogens10101292*
86. *Mc Clurkin A.W., Littledike E.T., Cutlip R.C., Frank G.H., Coria M.F. Bolin S.R. Production of cattle immunotolerant to bovine viral diarrhea virus. Canadian journal of comparative medicine 1984, 48(2):156-161.*
87. *Roeder P.L., Jeffrey M., Cranwell M.P. Pestivirus fethopathogenicity in cattle: changing sequale with fetal maturation. Veterinary Record 1986, 118(2):44-48. http://dx.doi.org/10.1136/vr.118.2.44*
88. *Han J.H., Weir A.M., Weston J.F., Heuer C., Gates M.C. Elimination of bovine viral diarrhoea virus in New Zealand: a review of research progress and future directions. New Zealand Veterinary Journal 2018, 66, 273–80, 2018. https://doi.org/10.1080/00480169.2018.1509030*
89. *Decaro N. HoBi-like pestivirus and reproductive disorders. Frontiers in Veterinary Science 2020, 7, 622447. https://doi.org/10.3389/fvets.2020.622447*
90. *Carman S., van Dreumel T., Ridpath J., Hazlett M., Alves D., Dubovi E., Tremblay R., Bolin S., Godkin A., Anderson N. Severe acute bovine viral diarrhea in Ontario, 1993-1995. Journal of Veterinary Diagnostic Investigation 1998, 10:27-35. https://doi.org/10.1177/104063879801000106*
91. *Bennett R.M., Done J.T. Control of BVDV: a case for social cost-benefit analysis? Proceedings of the Meeting of the Society of Veterinary epidemiology and Preventive Medicine, 2-4 April 1986, Edinburgh, pp 54-65.*
92. *Valle P.S., Skjerve E., Martin S.W., Larssen R.B., Østerås O., Nyberg O. A cost-benefit evaluation of the Norwegian bovine virus diarrhoea control and eradication program, Proceedings of the 9th Symposium of the International Society for Veterinary Epidemiology and Economics (ISVEE), 6-11 August 2000, Breckenridge, CO, USA, pp. 1189-1191.*
93. *Valle P.S. Ten years of bovine virus diarrhoea virus (BVDV) control – a beneficial joint effort in three parts, Second European Symposium on BVDV Control, 20-22 October 2004, Porto Portugal, Programme and Abstracts, p. 50.*
94. *Chi S., Chen S., Jia W., He Y., Ren L., Wang X. Non-structural proteins of bovine viral diarrhea virus. Virus Genes 2022, 58:491–500. https://doi.org/10.1007/s11262-022-01914-8*
95. *Workman A.M., Heaton M.P., Vander Ley B.L., Webster D.A., Sherry L., Bostrom J.R., Larson S., Kalbfleisch T.S., Harhay G.P., Jobman E.E., Carlson D.F., Sonstegard T.S. First gene-edited calf with reduced susceptibility to a major viral pathogen. PNAS Nexus 2023, 2(5), 125.https://doi.org/10.1093/pnasnexus/pgad125*
96. *Walz P.H., Chamorro M.F., Falkenberg S.M., Passler T., van der Meer F., Woolums A.R. Bovine viral diarrhea virus: An updated American College of Veterinary Internal Medicine consensus statement with focus on virus biology, hosts, immunosuppression, and vaccination. Journal of Veterinary Internal Medicine 2020, 34:1690–1706. https://doi.org/10.1111/jvim.15816*
97. *Belić L., Mihajlović B., Jermolenko G. Prilog ispitivanju antitela protiv virusne dijareje goveda. Veterinarski glasnik 1973, 8, 565-568*
98. *Wernike K., Schirrmeier H., Strebelow, H. G., Beer M. Eradication of bovine viral diarrhea virus in Germany—Diversity of subtypes and detection of live-vaccine viruses. Veterinary microbiology 2017, 208, 25-29.*
99. *Waage S., Krogsrud, J., Nyberg O. The Norwegian programme for eradication of bovine viral diarrhoea/mucosal disease, 1994.*
100. *Houe H. Economic impact of BVDV infection in dairies. Biologicals 2003, 31(2), 137-143.*
101. *Harkness J.W., Roeder P.L., Drew T., Wood L., Jeffrey M. Pestiuirus Infection of Ruminants. JW Harkness (ed). Brussels, CEC, 1987, p 233.*

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MONITORING TOPLOTNOG STRESA KOD KRAVA – STREMLJENJE KA BOLJOJ REZILIJENCIJI

MONITORING OF HEAT STRESS IN DAIRY COWS – STRIVING TOWARDS BETTER RESILIENCE

Mira Majkić[a], Jovan Spasojević[a], Sandra Nikolić[a], Marko Cincović[a]

[a]Departman za veterinarsku medicinu, Poljoprivredni fakultet, Univerzitet u Novom Sadu
*Autor za korespodenciju: miramajkic@gmail.com

M.M. https://orcid.org/0009-0007-0110-7327, J.S. https://orcid.org/0000-0001-5654-5796, S.N. https://orcid.org/0000-0001-7337-9937, M.C. https://orcid.org/0000-0002-5683-8983

SAŽETAK

Toplotni stres se može definisati kao stanje kod koga dolazi do promenjenog odnosa u količini proizvedene toplote i toplote koja se odaje u spoljašnju sredinu putem fizičkih modela kondukcije, konvekcije i evaporacije. Duže delovanje toplotnog stresa može negativno uticati na dravstveno-produktivne osobine. Adaptivni odgovor životinja na toplotni stres podrazumeva fiziološke, bihejvioralne ii metaboličke reakcije u cilju održavanja termolegulacije. Kao glavni efekat toplotnog stresa javlja se opadanje proizvodnje mleka, pa tako toplotni stres ima veliki ekonomski značaj. Opadanje proizvodnje mleka može biti u vezi sa direktnim delovanjem visokih ambijentalnih temperatura, ali i u vezi sa metaboličkim, endokrinološkim i imunološkim promenama, a svi oni zavise od načina ishrane, nege i bioloških predispozicija krava. Cilj ovog rada je da se prikaže kako svaki od navedeneih faktora utiče na proizvodnju mleka u toplotnom stresu i koje su to biološke osobine krava i zdravstvene i tehnološke mere koje možemo preduzeti u cilju povećanja rezilijentnosti krava na toplotni stres.

Ključne reči: krave, toplotni stres, adaptacija, odgovor, rezilijencija.

ABSTRACT

Heat stress can be defined as a condition in which there is a changed relationship in the amount of heat produced and heat that is released to the outside environment through physical models of conduction, convection and evaporation. Prolonged exposure to heat stress can negatively affect health-productive traits. The adaptive response of animals to heat stress involves physiological, behavioral and metabolic reactions in order to maintain thermoregulation. The main effect of heat stress is a decrease in milk production, so heat stress has great economic importance. The decrease in milk production can be related to the direct effect of high ambient temperatures, but also related to metabolic, endocrinological and immunological changes, all of which depend on the way of feeding, care and biological predispositions of cows. The aim of this paper is to show how each of the mentioned factors affects milk production in heat stress and what are the biological characteristics of cows and the health and technological measures that we can take in order to increase the resilience of cows to heat stress.

Key words: cow, heat stress, adaptation, response, resilience.

UVOD

Toplotni stres se može definisati kao stanje kod koga dolazi do promenjenog odnosa u količini proizvedene toplote i toplote koja se odaje u spoljašnju sredinu putem fizičkih modela kondukcije, konvekcije i evaporacije. Toplotni stres nastaje kao posledica dužeg delovanja visokih ambijentalnih temperatura i akumulacije viska toplote unutar tela.(1) Kao posledica delovanja

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toplotnog stresa dolazi do povećanja rektalne temperature, frekvence disanja i srčanog ritma. Toplotni stres se negativno odražava na proizvodnju i kvalitet mleka, produkciju, fertilnost i celokupni zdravstveni status. (2,3). Kao glavni indikator toplotnog stresa uzima se THI indeks. THI indeks se definiše kao odnos temperature vazduha izmerene u °C i relativne vlažnosti vazduha izmerene u %. Ns osnovu vrednosti THI indeksa topotni stres može biti blag, umeren jak i veoma jak. Visoke vrednosti THI indeksa utiču na kvalitet i količinu proizvedenog mleka uključujući mlečnu mast, mlečne proteine, laktozu i suvu materija u mleku.(4) Rrezilijencija predstavlja sposobnost nekog sistema da se prilagodi novonastalim uslovima u cilju održavanja homeostaze. Indikatori rezilijencije kod goveda kod toplotnog stresa mogu biti fiziološki (povećanje frekvence disanja i srčanog rada, smanjen unos hrane), lokomotorni (da se životinja prirodno pomeri na mesto gde je veći protok vazduha) i metabolički (promene u metabolizmu ugljenih hidrata, masti proteina, koje dovode do smanjnjea količine I promenjenog sastava mleka) (5). U cilju smanjneja efekata toplotnog stresa treba obezbediti adekvatne mikroklimatske uslove i podesiti modele ishrane novonastalim uslovima. Bitan faktor u cilju smanjneja štetnih efekata je genetska selekcija goveda na rase koje su otporne na toplotni stres (6).

METABOLIČKE PROMENE KAO OSNOV SMANJENE PROIZVODNJE MLEKA U TOPLOTNOM STRESU

Toplotni stres izaziva promene u sastavu metabolita koje ulaze u mlečnu žlezdu. Te promene uključuju promene u količini glukoze, laktoze, ketona, promene u metabolizmu aminokiselina i nukleotida. Krajnji efekat je taj da mlečna žlezda dobija manju količinu sirovina koje koristi za sintezu mleka, što za posledicu ima smanjenje produkcije i promene u njegovom sastavu (7). Metaboliti iz krvi i mleko (količina i sastav) mogu biti pogodni indikatori metaboličkog odgovora na toplotni stres, a mogu se identifikovati kao animalni biofluidi (serum, plazma, mleko, urin). Napravljeni su metabolički protokoli u cilju identifikacije odgovora na visoke temperature. Kod uzoraka mleka korišćena je nuklearna magnetna rezonanca (NMR), tečna i gasna hromatografija, masena spektormetrija (GC-MS/LC-MS). Smatra se da NMR može najpreciznije pružiti informacije o povezanosti metabolita, količine i sastava mleka (8).

U studiji Fan et al. (9), rađen je uticaj toplotnog stresa na energetski metabolizam kod Holštajn krava. Rezultati su pokazali pad koncnetracije glukoze, laktoze i galaktozo 1-fosfata,kao i povećanje koncentracije acetoacetata I β-hidroksibutirata (metaboliti ketona). Identifikovana su 34 metabolita mleka koji su uključeni u metabolizam aminokiselina, glukoze, laktoze. Promene u koncentraciji metabolita između krvi i mlečne žlezde može uticati na količinu sintetizovanih prekurzora u mlečnoj žlezdi. Rezultati studije poazauju da postoje biomarkeri koji se mogu koristiti u proceni toplotnog stresa. Analiza pomenutih biomarkera može pomoći u tumačenju pojedinih fizioloških mehanizama koje nastaju tokom toplotnog stresa a koji bi doveli do narušavanja zdravstvenog staja i laktacije

Rezultati Abeni i sar. (2007) (10) i O'brien i sar. (2010) (11) su pokzali da ekspozicija krava vrednostima THI indeksa iznad 72 negativno utiče na koncentraciju glukoze. Rezultati istrazaživanja su pokazali da je koncentracija glukoze tokom trajanja toplotnog stresa bila niža sa trendom daljeg pada. U rezultatima Baumgard i sar. (2013) (12) je koncentacija glukoze je bila za 7% niža tokom trajanja toplonog stresa i imala je dalju tendenciju pada, a do istog zaključka su došli Koubkova i sar. (2002) (13) U istraživanju Scharf i sar. (2010) (14)' najniže vrednosti glukoze bile su pri vrednostima THI indeksa od 80 – 85 (3,77 mmol/L). Na vrednostima <80 vrednosti su izmerene (3,96 mmol/L), dok je nivo glukoze na vrednostima THI >85 iznosio 3,90 mmol/L. U istraživanju Febbraio i sar. (2001) (15) koncentracija glukoze tokom toplotnog stresa iznosila je 71,78 mg/dl, dok je tokom termoneutralnog perioda iznosila 77,67 mg/dl.. Koncentracija glukoze u istraživanju Ikari i sar. (2005) (16) je iznosila 2,94 mmol/l. Slični rezultati prikazani su i u studiji Shwartz i sar. (2009) (17) gde je koncentracija glukoze tokom letnjeg perioda iznosila 2,73 mmol/L dok je tokom zimskog perioda ova vrednost bila 3,42 mmol/L. Najveće smanjenje glukoze je zabeleženo drugog dana po ekspoziciji. Koncentracija insulina bila je povećana (2mg / ml) dok je na početku ispitivanog perioda iznosila (1 mg / ml). Koncentracija NEFA bila je smanjena (1 mmol/L) a pre ekspozicije ova vrednost je iznosila (1,3 mmol/L)

Hipoglikemija koja je prisutna tokom toplotnog stresa može se objasniti povećanim

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nivoom glikogenolize sa jedne strane i smanjnjea glukoneogeneze u jetri sa druge strane. Tokom ekspozicije visokim ambijentalnim uslovima dolazi I do endokrinih izmena gde se kao posledica glukoza troši kaoizvor energije. Još jedan razlog za korišćenje glukoze kao osnovnog izvora eneroje je taj da se prilikom metabolizma glukoze oslobađa manja količina energije nego kada se izmetaboliše ista količina masti. Takođe je prisutan povećani absorptivni kapacitet glukoze iz creva kao i povećana reapsorpcija glukoze preko bubrega. (16,17)

Tokom ekspozicije visokim ambijentalnim temperaturama primećene je i drugačiji lipolitički odgovor masnog tkiva. Izmenjen odgovor nastaje usled aktivnosti specifičnih enzima (HSL – hormon senzitivna lipaza). Greenberg i sar., (2011) (18) su dokazali povećanu fosforilaciju HSL tokom trajanja toplotnog stresa. Aktivna forma enzima započinje lipolitički efekat odnosno hidrolizu triglicerida, što bi dovelo do pojačane lipolize. Međutim, u toplotnom stresu pankreas luči veću količinu insulina. Insulin predstavlja snažan antilipolitički hormon, pa se kao posledica njegovog delovanja sprečava lipoliza. Hiperinsulinemija predstavlja glavni adaptacioni mehanizam preko koga se glukoza usmerava na metabolisanje i time oslobađa manja količina energije (11).

Tokom ekspozicije visokim temperaturama primećena je povećana koncentracija kortizola. Povećanje koncentracija kortizola se značajno povećava u odnosu na početnu vrednost kod holštajn krava izloženih temperaturi od 42◦C tokom 160 min. Značajno je napomenuti da je koncentracija znatno opala unutar 5 min nakon prestanka izlaganja visokim temperaturama (20). Promene u proizvodnji isparljivih masnih kiselina (VFA) su takođe prisutne i toplotni stres je povećao proizvodnju propionata i butirata u odnosu na koncentraciju acetatata Nonaka et al., 2008 (20) su našli smanjenje koncentracije insulinskog faktora rasta-I (IGF-I), vitamina C u plazmi (21) i neesterifikovane masne kiseline (NEFA). Naveden metaboličke promene utiču da u mlečnu žlezdu pristiže manje prekurzora koji služe za sintezu mleka, što za posledicu ima smanjenje količine i sastava mleka.

Međutim životinje koje su izložene visokim temperaturama tokom određenog vremena imaju mogućnost da se na novonastale uslove adaptiraju i razviju adekvatan odgvor organizma. Promene u adaptaciji se mogu izraziti preko tzv. krive rezilijentnosti. Kod visokomlečnih krava koje su izložene visokim vrednostima THI rezlikujemo tri tipa rezilijentnih kriva. Prvi tip krive podrazumeva nagli pad u proizvodnji mleka, i kasniji postepeni oporavak. Drugi tip podrazumeva spontani pad u proizvodnji mleka i spontani oporavak nakon prestanka delovanja sresogenog faktora. Treći tip krive karakteriše lagani pad u proizvodnji mleka, ali brz oporavak nakon delovanja stresogenog faktora. Krive rezilijentnosti utiču na kumulativne gubitke mleka. Što je period od početka delovanja stresogenog faktora pa do postizanja najniže tačke u proizvodnji mleka duži, veći su I kumulativni gubici. Bitno je napomenuti da faaza laktacije utiče na krive rezilijentnosti. Kada se posmatraju metabolički parametri, razlikuju se četiri tipa rezilijentnih kriva u zavisnosti da li određeni metabolit opada ili raste. Prvi tip krive podrazumeva nagli porast ili pad metabolita nastao kao posledica delovanja stresogenog faktora. Nakon delovanja stresogenog faktora dolazi do brzog vraćanja na početne vrednosti, a zatim odlaska u suprotan smer. Drugi tip rezilijentnih kriva pokazuju metaboliti čija koncentracija naglo porasteili padne na početku delovanja stresogenog faktora, na tom nivou se održava izvesno vreme, da bi se nakon prestanka delovanja stresogenog faktora loncentracija metabolita brzo vratila na početnu vrednost. Treći tip rezilijentnih kriva pokazuju metabolite kod kojih dolazi do laganog padaili povećanja u koncentraciji na početku delovanja toplotnog stresa i laganog vraćanja na prvobitne vrednosti nakon prestanka delovanja stresogenog faktora. Četvrti tip krive predstavljau metabolite kod kojih dolazi do laganog opadanjaili porasta u koncentraciji. Prvi idrugi tip krive pokazuju najveći kumulativni efekat na vrednosti metabolita. Predikcija u proizvodnji mleka je mnogo preciznija kada se posmatra kumulativni efekat promene metaolita I kumulativni gubitak mleka nego kada se samo koristi uticaj koncentracije metabolita na količinu proizvedenog melka (22).

UTICAJ THI NA PROIZVODNJU MLEKA

THI se koristi kao idikator toplotnog opterećenja.U Indiji pri vrednostima THI indeksa iznad 72 gubici mlkea se kreću od 18 ± 1.4 do 10 ± 0.92 L (23). Prinos mleka je negativno povezan sa THI. Na Tajlandu, Sungkhapreecha et al. (24) su utvrdili manji pad u proizvodnji mleka 70–80 g po jedinici povećanja vrednosti THI od 76. U Keniji gubici mleka kreću se rasponu od 0.27, 0.18 and 0.35 kg/°C po danu u toku prve, druge I treće

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laktacije pri vrednostima THI indeksa od 72. (25). U brazilu sus e gubici u proizvodnji mleka kretali u rasponu od 0,094 kg/danu za vrednosti THI indeksa iznad 69 dok su pri vrednostima THI indeksa iznad 66 gubici bili 0,23 kg/danu. Količina mleka se samnjuje za 0,5 kg/danu I više pri vrednosti THI indeksa od 76.. (26)

Nekoliko studija je pokazalo negativnu korelaciju između proizvodnje mleka I THI (27,28), posebno kada dnevni prosek THI prevaziđe vrednost od 68. Rezultati u (29) s slažu sa rezultatima predhodnih autora gde je povećana vrednost THI indexa dovela do većeg gubitka mleka pogotovu kod visoko produktivnih krava. Pored toga,primećen je smanjen prinois mleka, ali tri do pet dana nakon ekspozicije visokim temepraturama (30,31). Rezultati. (32) su pokazali da je najveća prosečna mlečnost dobijena u oktobru, dok je najniži prinos zabeležen u julu, čime se potvrđuje inverzna korelacija između prinosa mleka i efekata toplotnog stresa preko modela sa vrednostima THI. Do sličnih rezultat su došli (33) Primećeno je da su mlečna govedakoja su bila izložena visokim temperaturama i redukovanom unosu hrane imala za oko 20% manju proizvodnju mleka u odnosu na grupu koja je bila pod termoneutralnim uslovima. U studiji (34) je primećeno da je kod goveda kod kojih je muža započinjana u najtoplijrm periodu godine količina mleka bila znatno niža u odnosu na kolkičinu mlkea koja se dobija pri muži u termoneutralnim uslovima. Istraživanje sprovedeno u studiji (35) je pokazal da ukoliko dođe do povećanja telesne temperature kod gravodnih krava količina mleka koja se dobija tokom naredne tri laktacije je znatno manja. Faza laktacije je presudan faktor u proceni uticaja toplotnog stresa obzirom da krave srednje laktacije pokazuju veću osetljivost u poređenju sa kravama u ranoj i kasnoj fazi laktacije. Pored toga, tokom rane faze laktacije, proizvodnja mleka kod krava se u velikoj meri održava mobilizacijom masti iz masnih depoa i manjim obimom unosa hrane. Nasuprot tome, u fazi srednje laktacije, prinos mleka prvenstveno zavisi od unosa hrane (36). Toplotni stres ne utiče samo na količini proizvedenog mleka nego i na sastav dovodeći do smanjenja nivoa proteina, masti i laktoze

Nekoliko istraživanja (37, 38) je potvrdilo je jaku korelaciju između THI i procenta i mlečne masti i mlečnih proteina. Studija (39) je primetila smanjenje u koncentraciji masti i proteina u mleku tokom letnjih meseci, dok je povećanje u koncentraciji primećeno tokom zimske sezoneSezona nije pokazala uticaj na količinu laktoze.Međutim, postoje istraživanja koja su potvrdila suprotne rezultate (40) Laktoza je povezana sa proizvodnjom α-La, koenzima koji je neophodan za njeno formiranje. Ovaj specifični protein surutke se sintetiše u mlečnim žlezdama gde α-La interaguje sa β-1,4-galaktoziltransferazom u goldžijevom aparatu epitelnih ćelija mlečne žlezde kako bi se olakšalo formiranje enzima laktoza-sintaza. α-La menja specifičnost supstrata β-1,4-galaktoziltransferaze sposobnost sinteze laktoze iz glukoze i UDP-galaktoze. Mlečna mast je neophodna za formiranje emulzuje i deluje kao stabilizator. Rezultati (41) su pokazali smanjen sadržaj polarnih lipida od 43% nakon izlaganja toplotnom stresu u trajanju od dva dana. Nakon ekspozicije visokim temperaturama u trajanju od četiri dana, koncentracija lipida je opala za 52%. Nakon ekspozicije visokim THI vrednostima u trajanju od pet dana koncentracija lipida se vratila na početne vrenosti. Kratkotrajno izlaganje krava visokim vrednostima THI ne utiče na sastva proteina u mleku. Da bi se koncentracija proteina smanjila potrebno je da krave budu izložene uzastopno tokom najmanje tri dana. Razlog za smnjenje koncentracije proteina u mleku tokom ekspozicije visokim temperaturama može se objasniti njihovim specifičnim smanjejem u mlečnoj žlezdi. Toplotni stres dovodi I do smanjenja masene frakcije αS1-kazeina i smanjenja αS2-kazeina. Visoke temperature dovode i do smanjneja ukupne količine aminokiselina (esencijalne i neesenzijalne) za 17,1%

GENETIČKA SELEKICJA GOVEDA I TOPLOTNI STRES

Postoje rase goveda koje su prirodno otporne na visoke temperature. Sa povećanjem temperature vazduha evaporativni odgovor kod krava Jerzej rase je linearno porastao, dok su goveda tipa Bos indikus reagovala tek kada je temperatura dostigla vrednosti 30 i 35◦C. Studija (42) koja je rađena u Australiji je imala za cilj da ispita kako različite rase goveda reaguju na visoke i niske temperature. Posmatrana su goveda Frizijskog tipa, Bramansko goveče (američka vrsta zebu goveda hibridnog tipa) i linija nastala ukrštanjem Frizijskog i Bramanskog tipa goveda. Životinje su izlagane temperaturi od 17,2°C, i temepraturi od 37.8°C. Rezultati su pokazali das su Frizijske rase goveda osetljivje na toplotni stres. Najveće povećanje frekvence disanja i rektalne temperature zabeleženo

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je kod goveda Frizijske rase. Međutim, kada se međusobno porede goveda Angus rase i Bramansko goveče, otpornost na visoke temperature zabeležena je kod Bramanskog tipa goveda. Ukrštena Šarole goveda su imala nešto više rektalne temperature (39.9., 39.6 ± 0.04◦C) u odnosu na ukrštena Angus goveda. (43). Goveda Bos indicus tipa pokazuju bolju adaptaciju (brzina metabolizma, potrošnja hrane) u odnosu na goveda Bos taurus tipa. Generalno posmatrano tropske rase goveda se smatraju tolerantnijm na visoke temperature nego što bi bile rase koje naseljavaju umereno topla klimatska područja. Međutim postoje i određene interindividualne varijacije koje utiču na otpornost na visoke temperature. Boja dlačnog pokrivača utiče na tolotnu osetljivost. Smatra se da su goveda sa tamnijim dlačnim pokrivačem osetljivija na topltni stres. Goveda *Bos taurus* tipa sa tamno-crvenim dlačnim pokrivačem imaju veću toplotnu kondukciju za 0,3 stepena u odnosu na drug erase, pa se smatraju osetljivijom kategorijom. Goveda sa crnom bojom dlačnog pokrivača su imali za 5,6 - 11,7◦C višu temperaturu tela u poređenju sa govedima koja imaju belu boju dlačnog pokrivača. Drugi mogući izvori varijacija mogu nastati zbog metaboličkih i fizioloških razlika između životinja otpornih na toplotu. Gaughan et al. (2010) su potvrdili da je identifikacija toplotno tolerantnih pojedinaca u okviru visokoproduktivnih rasa korisna procena otpornosti i opstanka pri visokim temperaturama. Povećana potražnja za goveđim mesom dovela je do toga da se ukrštanjem stvore rase koje će biti otporne na visoke temperature uz istovremeno očuvanje proizvodnih karakteristika. Međutim, takve linije još uvek nisu postignute, zato što rase goveda koje su otporne na visoke temperature često imaju lošije proizvodne rezultate. Ortiz-Colón et al. (2018) su došli do zaključka da se ukrštanjem holštajna sa Senopolskom rasom goveda dobija linija koja ima veću otpornost na visoke temperature. Zbog sve češćih klimatskih promena novija istraživanja bi trebala da budu usmerena na proizvodnju linija koje su otporne na visoke ambijentalne temperature.

KAKO SMANJITI TOPLOTNI STRES?

Fidler i sar. (44) su preporučili četiri koraka da bi se efekti toplotnoh stresa sveli na minimum. Prvi korak je obezbediti dovoljnu količinu hladne higijenski ispravne vode. Kada je vlažnost vazduha iznad 80% potreba za količinom vode je povećana za 50 %. Osim zadovoljenja potreba u smislu kvantiteta, potrebno je voditi računa o tome da temperatura vode, koja se pije bude prilagođena. Osim toga, sistem za napajanje treba da se nalazi u neposrednoj blizini životinje, na hladnom mestu, ukoliko se životinja drži u slobodnom sistemu, odnosno na razdaljini od 15 metara kod vezanog sistema držanja. Kod grupnog držanja životinja, potrebno je obezbediti najmanje dva napajališta. Ako se npr. uzme grupa od 100 krava, potrebno je obezbediti napajalište na svakih 3 metra razdaljine. Protok vode bi trebalo da bude minimum 3-5 litara po minuti, minimalna dubina korita 0,08 m. Površina korita po kravi treba da iznosti najmanje 0,65 kvadratnih metara, i da bude odignuta od zemlje 1,2 metara. Drugi korak podrazumeva postavljanje ventilatora u objektu, kao i postavljanje prskalica, koje izbacuju određenu količinu vode na određeni vremenski period. Međutim, sa prskalicama treba biti obazriv, jer prevelika količina vode u zagrejanom objektu može onemogućiti mehanizme za odavanje toplote. Takođe je potrebno voditi računa o poziciji ventiltora kod vezanog sistema držanja. Uloga ventilatora je u većem protoku hladnog vazduha, koji pospešuje procese rashlađivanja. Stoga je poželjno postaviti dva ventilator ispred mesta vezivanja, i dva odprilike na pola dužine veza. Kada su upitanju prskalice, da bi se životinja rashladila, potrebno je minimum 25 litara vode. Kod ovakvog načina rashlađivanja potrebno je voditi računa o tipu poda (da nije klizav, i da se višak vode može eliminisati, zato što se veća količina vode pri kontaktu sa akropodijumom negativno odražava na njegovo zdravstveno stanje). Treći korak podrazumeva postavljanje prskalica neposredno ispred hranilica. Kod ove procedure neophodno je da se hrana pokrije (kako bi se sprečio kvar). Ovaj sistem omogućava da životinja ima dostupnu rashlađenu hranu, koju če radije konzumirati. Četvrti korak podrazumeva korekciju ishrane. Krave tokom letnjih meseci uzimaju manju količinu hrane zbog kalorigenog efekta, ali se smanjenjem suve materije hrane ovaj problem može prevazići. Ishrana sa manje, kao i ishrana visokokvalitetnim zrnastim hranivima doprinosi manjem opterećenju tokom letnjih meseci. Kod ovakvog režima ishrane neophodno je krigovati mineralni balans (kalijim 1,3-1,5 %, natrijum 0,5-0,6 % , magneziijum 0,3-0,4 %). Hlor treba da bude zastupljen u koncentraciji od 0,25%, a procenat razgradivih proteina ne bi trebalo da prelazi 65%. Primenom protektivnih mera moguće je smanjiti negativne efekte topltnog stresa. Konkretno, obezebeđivanjem adekvatnog mikroklimata, moguće je smanjiti štetne efekte za 89% Genetska selekcija na rase koje bi bile otporne na visoke temperature može imati pozitivne efekte kada se posmatra na duži vremenski period. Obzirom da postoje interindividualne varijacije potrebno je razmotriti genotipske karakteristike, zdravstveni status, adaptivne mehanizme kao I eksterijerne osobine, na individualnom nivou.

ZAKLJUČAK

Toplotni stres može dovesti do značajnih ekonomskih gubitaka u stočarskoj proizvodnji Praćenje toplotnog stresa kao i uvođenje mera za njegovo ublažavanje su se ranije zasnivale na praćenju odgovora pojedinih životinja na novonastale uslove. Obzirom da je individualno praćenje odgovora na stres nepraktično za večće komercijalne farme, potrebno je razviti univerzalni sistem praćenja stepena toplotne opterećenosti u realnom vremenu. Automatsko upravljanje toplotnim stresom goveda uz korišćenje uređaja koji kontrolišu lokaciju i kretanje (upotreba GPS-a) bi olakšali detekciju stres osetljivih jedinki u grupi. Značaj vizuelnih posmatranja i indeksa baziranih na vremenskim prilikama se svakako ne može isključiti zato što ovakvi modeli mogu da se primene na različite proizvodne osobine i različite vrste životinja. Toplotni talasi su poslednjih godina sve češći, pa je dugoročno potrebno razviti mere koje bi negativne efekte toplotnog stresa svele na minimum, a proizvodnju učinile održivom.

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LITERATURA

1. *Bernabucci U., Biffani S., Buggiotti L., Vitali A., Lacetera N., Nardone A. The effects of heat stress in Italian Holstein dairy cattle. J Dairy Sci. 2014, 97:471–86. doi: 10.3168/ jds.2013-661*
2. *Corazzin M., Saccà E., Lippe G., Romanzin A., Foletto V., Da Borso F., et al. Effect of heat stress on dairy cow performance and on expression of protein metabolism genes in mammary cells. Animals 2020, 10:1–13. doi: 10.3390/ani10112124*
3. *Marumo J.L., Lusseau D., Speakman J.R., Mackie M., Hambly C. Influence of environmental factors and parity on milk yield dynamics in barn-housed dairy cattle. J Dairy Sci. 2022, 105:1225–41. doi: 10.3168/jds.2021-20698*
4. *Dauria B.D., Sigdel A., Petrini J., Bóscollo P.P., Pilonetto F., Salvian M., et al. Genetic effects of heat stress on milk fatty acids in Brazilian Holstein cattle. J Dairy Sci. 2022, 105:3296–305. doi: 10.3168/jds.2021-2091*
5. *Galán E., Llonch P., Villagrá A., Levit H., Pinto S., Del Prado A. A systematic review of non-productivity-related animal-based indicators of heat stress resilience in dairy cattle. PloS one 2018, 13(11), e0206520.*
6. *Habimana V., Nguluma A.S., Nziku Z.C., Ekine-Dzivenu C.C., Morota G., Mrode R., Chenyambuga S.W. Heat stress effects on milk yield traits and metabolites and mitigation strategies for dairy cattle breeds reared in tropical and sub-tropical countries. Frontiers in Veterinary Science 2023, 10, 1121499.*
7. *Fan C., Su D., Tian H., Li X., Li Y., Ran L., et al. Liver metabolic perturbations of heatstressed lactating dairy cows. Asian-Austr J Anim Sci. 2018, 31:1244–51. doi: 10.5713/ ajas.17.0576*
8. *Yue S., Ding S., Zhou J., Yang C., Hu X., Zhao X., et al. Metabolomics approach explore diagnostic biomarkers and metabolic changes in heat-stressed dairy cows. Animal 2020, 10, 10, 1741. https://doi.org/10.3390/ani10101741*
9. *Fan C.Y., Su D., Tian H., Hu R.T., Ran L., Yang Y., et al. Milk production and composition and metabolic alterations in the mammary gland of heat-stressed lactating dairy cows. J Integ Agric 2019, 18, 12, 2844-2853*
10. *Abeni, F., Calamari, L., Stefanini, L. Metabolic conditions of lactating Friesian cows during the hot season in the Po valley. 1. Blood indicators of heat stress. International journal of biometeorology 2007, 52, 2, 87-96*
11. *O'brien M.D., Rhoads R.P., Sanders S.R., Duff G.C., Baumgard L.H. Metabolic adaptations to heat stress in growing cattle. Domestic animal endocrinology 2010, 38(2), 86-94.*
12. *Baumgard L.H., Rhoads Jr R.P. Effects of heat stress on postabsorptive metabolism and energetics. Animal. Review of Animal Biosciences 2013, 1(1), 311-337*
13. *Koubkova M., Haertlova H., Knizkova I., Kunc P., Flusser J., Dolezal O. Influence of high environmental temperatures and evaporative cooling on some physiological, hematological and biochemical parameters in high-yielding dairy cows. Czech Journal of Animal Science 2002, 47(8),309-318.*

ISBN 978-86-7520-611-8

14. *Scharf B., Carroll J.A., Riley D.G., Chase Jr C.C., Coleman S.W., Keisler D.H., Spiers D.E. Evaluation of physiological and blood serum differences in heat-tolerant (Romosinuano) and heat-susceptible (Angus) Bos taurus cattle during controlled heat challenge. Journal of animal science 2010, 88(7), 2321-2336*
15. *Febbraio M.A. Alterations in energy metabolism during exercise and heat stress. Sports medicine 2001, 31(1), 47-59.*
16. *Ikari A., Nakano M., Suketa Y., Harada H., Takagi K. Reorganization of ZO-1 by sodium-dependent glucose transporter activation after heat stress in LLC-PK1 cells. Journal of cellular physiology 2005, 203(3), 471-478.*
17. *Shwartz G., Rhoads M.L., VanBaale M.J., Rhoads R.P., Baumgard L.H. Effects of a supplemental yeast culture on heat-stressed lactating Holstein cows1. Journal of Dairy Science 2009, 92(3), 935-942*
18. *Greenberg A.S., Kraemer F.B., Soni K.G., Jedrychowski M.P., Yan Q.W., Graham C.E., Bowman T.A., Mansur A. Lipid droplet meets a mitochondrial protein to regulate adipocyte lipolysis. The EMBO journal 2011, 30: 4337-4339.*
19. *Abilay T.A., Mitra R., Johnson H.D. Plasma cortisol and total progestin levels in Holstein steers during acute exposure to high environmental temperature (42C) conditions. J. Anim. Sci. 1975, 41, 113–117*
20. *Nonaka I., Takusari N., Tajima K., Suzuki T., Higuchi K., Kurihara M. Effects of high environmental temperatures on physiological and nutritional status of prepubertal Holstein heifers. Livest. Sci. 2008, 113, 14–23.*
21. *Padilla L., Matsui T., Kamiya Y., Kamiya M., Tanaka M., Yano H. Heat stress decreases plasma vitamin C concentration in lactating cows. Livest. Sci. 2006, 101, 300–304.*
22. *Majkić M., Cincović M., Spasojević J., Jožef I., Blond B., Kovačević D. Kriva rezilijentnosti i kumulativni odgovor krava na toplotni stres. Letopis naučnih radova, 2023.*
23. *Kohli S., Atheya U.K., Thapliyal A. Assessment of optimum thermal humidity index for crossbred dairy cows in Dehradun district, Uttarakhand. India Vet World. 2014, 7:916–21.*
24. *Sungkhapreecha P., Chankitisakul V., Duangjinda M., Buaban S., Boonkum W. Determining heat stress effects of multiple genetic traits in tropical dairy cattle using single-step genomic BLUP. Vet Sci. 2022, 9:1–13.*
25. *Mbuthia J.M., Mayer M., Reinsch N. Modeling heat stress effects on dairy cattle milk production in a tropical environment using test-day records and random regression models. Animal 2021, 15:100222. doi: 10.1016/j.animal.*
26. *Santana M.L., Bignardi A.B., Pereira R.J., Stefani G., El Faro L. Genetics of heat tolerance for milk yield and quality in Holsteins. Animal 2017, 11:4–14.*
27. *Kino E., Kawakami R., Minamino T., Mikurino Y., Horii Y., Honkawa K., Sasaki Y. Exploration of Factors Determining Milk Production by Holstein Cows Raised on a Dairy Farm in a Temperate Climate Area.* Trop. Anim. Health Prod. 2019, 51, *529–536*
28. *Ekine-Dzivenu C.C., Mrode R., Oyieng E., Komwihangilo D., Lyatuu E., Msuta G., Ojango J.M.K., Okeyo, A.M. Evaluating the Impact of Heat Stress as Measured by Temperature-Humidity Index (THI) on Test-Day Milk Yield of Small Holder Dairy Cattle in a Sub-Sahara African Climate. Livest. Sci. 2020, 242, 104314*
29. *Summer, A., Lora, I., Formaggioni, P., Gottardo F. Impact of Heat Stress on Milk and Meat Production. Anim. Front. 2019, 9, 39–46.*
30. *Heinicke J., Ibscher S., Belik V., Amon T. Cow Individual Activity Response to the Accumulation of Heat Load Duration. J. Therm. Biol. 2019, 82, 23–32.*
31. *Wildridge A.M., Thomson P.C., Garcia S.C., John A.J., Jongman E.C., Clark C.E.F., Kerrisk K.L. Short Communication: (2018), The Effect of Temperature-Humidity Index on Milk Yield and Milking Frequency of Dairy Cows in Pasture-Based Automatic Milking Systems. J. Dairy Sci 2018, 101, 5, 4479-4482.*
32. *Reyad M.A., Sarker M.A.H., Uddin M.E., Habib R., Rashid M.H.U. Effect of Heat Stress on Milk Production and Its Composition of Holstein Friesian Crossbred Dairy Cows. Asian J. Med. Biol. Res. 2016, 2, 190–195.*
33. *Cowley F.C., Barber D.G., Houlihan A.V., Poppi D.P. Immediate and Residual Effects of Heat Stress and Restricted Intake on Milk Protein and Casein Composition and Energy Metabolism. J. Dairy Sci. 2015, 98, 2356–2368*
34. *Mellado M., Coronel F., Estrada A., Ríos F.G. Lactation Performance of Holstein and Holstein x GYR Cattle under Intensive Condition in a Subtropical Environment. Trop. Subtrop. Agroecosyst. 2011, 14, 3.*
35. *Laporta J., Ferreira F.C., Ouellet V., Dado-Senn B., Almeida A.K., De Vries A., Dahl G.E. Late-Gestation Heat Stress Impairs Daughter and Granddaughter Lifetime Performance. J. Dairy Sci. 2020, 103, 7555–7568*
36. *Bernabucci U., Lacetera N., Baumgard L.H., Rhoads R.P., Ronchi B., Nardone A. Metabolic and Hormonal Acclimation to Heat Stress in Domesticated Ruminants. Animal 2010, 4, 7, 1167-1183.*
37. *Das R., Sailo L., Verma N., Bharti P., Saikia J., Imtiwati, Kumar, R Impact of Heat Stress on Health and Performance of Dairy Animals: A Review. Vet. World 2016, 9, 260–268.*
38. *Mylostyvyi R., Chernenko O. Correlations between Environmental Factors and Milk Production of Holstein Cows. Data 2019, 4, 103.*

ISBN 978-86-7520-611-8

39. *Florio M., Giannone C., Ianni A., Bennato F., Grotta L., Martino, G. Seasonal and Feeding System Effects on Qualitative Parameters of Bovine Milk Produced in the Abruzzo Region (Italy). Agriculture 2022, 12(7), 917; https://doi.org/10.3390/agriculture12070917*
40. *Farrell H.M., Jimenez-Flores R., Bleck G.T., Brown E.M., Butler J.E., Creamer L.K., Hicks C.L., Hollar C.M., Ng-Kwai-Hang K.F., Swaisgood H.E. Nomenclature of the Proteins of Cows' Milk-Sixth Revision. J. Dairy Sci.2004, 87, 1641–1674.*
41. *Liu Z., Ezernieks V., Wang J. Wanni Arachchillage, N.; Garner, J.B.; Wales, W.J.; Cocks, B.G.; Rochfort, S. Heat Stress in Dairy Cattle Alters Lipid Composition of Milk. Sci. Rep. 2017, 7, 961.*
42. *Colditz P.J., Kellaway R.C. The effect of diet and heat stress on feed intake, growth, and nitrogen metabolism in friesian, F1 Brahman x Friesian, and Brahman Heifers. Aust. J. Agric. Res. 1972, 23, 717–725. doi: 10.1071/AR97 20717*
43. *Mitlohner F.M., Morrow J.L., Dailey J.W., Wilson S.C., Galyean M.L., Miller M. Shade and water misting effect on behavior, physiology, performance and carcass traits of heat-stressed feedlot cattle. J. Anim. Sci. 2001, 79,*
44. *Fidler A.P., VanDevender K. Heat Stress in Dairy Cattle. Cooperative Extension Service, University of Arkansas, US Department of Agriculture, and county governments cooperating, 2013.*

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MATHEMATICAL MODELING AND MACHINE LEARNING PREDICTION FOR PREVALENCE DYNAMICS OF CLINICAL MASTITIS IN DAIRY HERDS

MATEMATIČKO MODELIRANJE I PREDVIĐANJE MAŠINSKOG UČENJA ZA DINAMIKU PREVALENCIJE KLINIČKOG MASTITISA U MLEČNIM STADAMA

Dimitar Nakov[a*], Biljana Zlatanovska[b], Mirjana Kocaleva Vitanova[b], Marija Miteva[b], Slavča Hristov[c], Branislav Stanković[c]

[a]Faculty of Agriculture, Goce Delcev University, Stip, North Macedonia,
[b]Faculty of Computer Science, Goce Delcev University, Stip, North Macedonia
[c]Faculty of Agriculture, University of Belgrade, Belgrade-Zemun, Serbia
*Corresponding author: dimitar.nakov@ugd.edu.mk

D.N. https://orcid.org/0000-0001-6159-5595, B.Z. https://orcid.org/0000-0003-4300-2877, M.K.V. https://orcid.org/0000-0002-2444-2917, M.M. https://orcid.org/0000-0001-5326-2301, S.H. https://orcid.org/0000-0002-1719-9971, B.S. https://orcid.org/0000-0003-3925-6102

ABSTRACT

Mastitis remains one of the major diseases in dairy herds, causing profound economic losses to the entire milk production chain. The main aim of the study was an application of mathematical models and machine learning algorithms for the prediction of mastitis transmission in the dairy cow population. Data used for mathematical models and machine learning algorithms were obtained in a cross-sectional longitudinal survey lasting for one year by analyzing data for clinical mastitis occurrence in three dairy herds. For data prediction, simple SIR and SIRS mathematical models without vital dynamics and Weka software were applied. The annual prevalence rate of clinical mastitis for the entire population of cows was 34.13% on the cow level, 30.07% on the lactation level, while lactation incident risk was 45.86%. Most of the cows manifested one (68.24%) or two (18.63%) cases of clinical mastitis during lactation. The SIR model revealed that after a short time, the epidemic will disappear. From the explanation and the graphical presentations, it can be concluded that the stable point DFE attracts the trajectories of the system. The mastitis on the farms is calming down, and with these parameters of the model, an epidemic cannot occur. With the use of the decision table as one of the most used classification rules and cross-validation folds 10 we can best predict mastitis occurrence in dairy farms. Implementation of a good mastitis prevention program in dairy herds by increasing the rates of control parameters will reduce the mastitis pathogens transmission rates leading to a reduction of mastitis incidence.

Key words: mastitis, dairy cows, SIR, SIRS, machine learning

SAŽETAK

Mastitis je jedna od najznačajnijih bolesti u mlečnim stadama, koji izaziva velike ekonomske gubitke u celom lancu proizvodnje mleka. Osnovni cilj rada je bila primena matematičkih modela i algoritama mašinskog učenja za predviđanje prenošenja uzročnika mastitisa u populaciji muznih krava. Podaci, korišćeni za matematičke modele i algoritme mašinskog učenja, su dobijeni u longitudinalnom istraživanju poprečnog preseka u trajanju od godinu dana analizom podataka o kliničkoj pojavi mastitisa u tri mlečna stada. Za predviđanje podataka primenjeni su jednostavni SIR i SIRS matematički modeli bez vitalne dinamike i Veka softver. Godišnja stopa prevalencije kliničkog mastitisa za celokupnu populaciju krava iznosila je 34,13% na nivou krava, 30,07% na nivou laktacije, dok je rizik pojave u laktaciji iznosio 45,86%. Većina krava je u toku laktacije ispoljila jedan (68,24%) ili dva (18,63%) slučaja kliničkog mastitisa. SIR model je otkrio da će nakon kratkog vremena epidemija mastitisa nestati. Iz objašnjenja i grafičkih prikaza

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može se zaključiti da stabilna tačka DFE privlači putanje sistema. Mastitis na farmama se smiruje, a sa ovim parametrima modela ne može doći do epidemije. Primenom tabele odluka kao jednog od najčešće korišćenih klasifikacionih pravila i preklopa za unakrsnu validaciju 10 može se najbolje predvideti pojava mastitisa na farmama mlečnih krava. Sprovođenje dobrog programa prevencije mastitisa u mlečnim stadima povećanjem stope kontrolnih parametara će smanjiti stope prenošenja patogenih mikroorganizama uzročnika mastitisa, što će dovesti do smanjenja incidencije mastitisa.

Ključne reči: mastitis, mlečne krave, SIR, SIRS, mašinsko učenje

1.INTRODUCTION

Mastitis continues to be a significant concern within dairy herds, leading to considerable economic ramifications throughout the milk production chain. The dairy industry experiences substantial losses primarily attributable to decreased milk production and compromised milk quality (1,2). National statistics from the leading milk-producing European countries indicate that each year, 30 to 40% of dairy cows exhibit clinical signs of mastitis during the lactation period (3,4).
Mastitis is inflammation of the mammary gland that more often strikes cows with high levels of milk production, significantly impacting their milk yield (1). This condition not only affects the immediate productivity of dairy cows but also limits their genetic potential for milk production. The severity of milk yield reduction due to clinical mastitis (CM) varies based on the cow's number of lactations and the timing of the disease's onset during the lactation period (5). The most substantial decreases in milk production are typically seen when CM occurs early in the lactation period (6), with affected cows generally unable to achieve their expected milk yield for the remainder of that lactation period.
However, cases of CM represent just a tip of the total mastitis incidents. In fact, for every instance of clinical mastitis, there are 20 to 40 times more cases of subclinical mastitis (7). These subclinical cases may either evolve into CM or linger unnoticed for an extended duration. The emergence of mastitis within dairy herds is the result of a complex interaction between the cows, their environment, and various pathogens. Risk factors for CM in dairy operations are typically categorised into two main groups: those related to individual cows and those associated with environmental conditions (8,9,10,11).
The prevalence of udder pathogens responsible for both subclinical and clinical mastitis exhibits significant variation (12). Historically, primary udder pathogens in dairy herds included *Streptococcus agalactiae* and *Staphylococcus aureus*. However, with the adoption of modern milking practices and the implementation of mastitis control programs, the prevalence of these organisms has notably declined across many contemporary dairy farms. Presently, the common environmental pathogens encompass Coagulase Negative *Staphylococci* (CNS), *Streptococcus uberis, Streptococcus dysgalactiae*, *Klebsiella spp.*, and *Escherichia coli* (13,14).
Research efforts focused on developing decision-support tools for mastitis detection and management in dairy herds are ongoing. Mathematical modelling serves as a critical tool in comprehending and addressing mastitis within these herds (15). Additionally, understanding the pathways through which mastitis pathogens are transmitted is essential for making proper management decisions.
This paper discusses mathematical models framed within the broader concept of the epidemic structural equation models, focusing on SIR (Susceptible, Infected, Recovered) and SIRS (Susceptible, Infected, Recovered, Susceptible) frameworks to analyze the binary value of mastitis in dairy cows, making distinguish between healthy cows and those afflicted with clinical mastitis. These models intend to outline the causal, whether simultaneous or sequential, relationships among phenotypes, a common occurrence in various biological systems. For instance, in dairy cattle, a correlation exists where high milk production may lead to an increased risk of mastitis, which, in turn, negatively impacts milk yield.
In the realm of mathematical modelling, distinctions are made between SIR and SIRS models that do not incorporate vital dynamics (not considering rates of newly involved heifers and culling dairy cows) and those that do. This paper focuses on the application of SIR and SIRS models that exclude vital dynamics. These models categorize the population into three primary groups: Susceptible (S), Infected

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(I), and Recovered (R), aiming to predict the transmission dynamics of clinical mastitis within dairy herds. Through the analysis of these models, the paper also seeks to recommend strategies for a mastitis control program aimed at dairy herds, contributing to both theoretical and practical advancements in managing mastitis.
Furthermore, the paper utilizes machine learning (ML), a subfield of artificial intelligence (AI) dedicated to crafting algorithms and models that empower computers to learn from data autonomously and make predictions or decisions without explicit programming. ML finds application across diverse domains, including epidemiology, image and speech recognition, natural language processing, recommendation systems, healthcare, finance, and beyond. Within epidemiology, ML techniques are applied for disease surveillance, diagnostic and prognostic modelling, genomic epidemiology, risk factor identification, and various other scenarios.

2. MATERIALS AND METHODS

2.1. Epidemiological data

This study was conducted across three dairy farms situated in North Macedonia. Data were collected through a cross-sectional longitudinal survey spanning one year, focusing on the occurrence of clinical mastitis among milking cows. Each milking cow's yearly observation period began at a specific point in time and continued for one year, or from the start of the cow's lactation period within the observed timeframe until the finishing of lactation or the time point when the cow was removed from the herd. Consequently, a total of 1031 cows of the black-white breed, encompassing 1267 lactations, were monitored throughout the study duration.
The incidence of new cases of clinical mastitis was recorded daily throughout the research trial, using standard clinical observation under typical field conditions. Diagnosis of clinical mastitis involves a clinical examination of the udder (assessing for swelling, firmness, colour, pain, and impaired function) and evaluation of milk abnormalities (including the presence of watery milk, flakes, clots, blood, pus, or discolouration). The occurrence of clinical mastitis was quantified as prevalence per cow and lactation.

$$prevalence\ rate\ per\ cow\ (\%) = \frac{number\ of\ cows\ with\ clinical\ mastitis}{total\ number\ of\ observed\ dairy\ cows} x100$$

$$prevalence\ rate\ per\ lactation\ (\%) = \frac{number\ of\ lactation\ with\ case\ of\ clinical\ mastitis}{total\ number\ of\ observed\ lactation} x100$$

Lactation incidence risk (LIR) was calculated using the density method:

$$LIR\ (\%) = \frac{total\ number\ of\ clinical\ mastitis\ cases}{total\ number\ of\ observed\ lactation} x100$$

During the same lactation, a nine-day interval was used to make a distinction between two successive cases of clinical mastitis. This interval comprised four days of antibiotic treatment administered to the affected quarters of the mammary gland, followed by an additional four days during which the antibiotics remained present in the milk. Throughout this period, the milk was waved aside. Finally, on the ninth day, any abnormal changes in the milk were assessed to confirm the presence of mastitis symptoms (16).
Related to the mastitis status, dairy cows were allocated into three classes: the cows at risk of developing a clinical form of mastitis, cows that suffer from clinical mastitis and the class of dairy cows that have recovered from the mastitis and got temporary immunity.

2.2. Mathematical models

The SIR and SIRS frameworks were applied as predictive models for understanding the spread of mastitis within dairy cow populations. These models operate under the assumption of stable population size, denoted as N, thereby excluding vital dynamics such as the introduction of new heifers, culling, or deaths within the dairy cow population. Such models are referred to as closed epidemic models. Due to the infectious nature of mastitis, it is presumed that the entire milking cow population is at risk of infection. Cows exhibiting symptoms of clinical mastitis act as the infection's source, facilitating its spread within the herd. Typically, around nine days after showing signs of clinical mastitis, cows are again at risk of developing the disease.
Thus, the SIR model assumed that cows once recovered from mastitis cannot be reinfected, while the SIRS model posits that recovered cows remain

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susceptible and can indeed contract mastitis once more. The graphical visualizations of mathematical models were made in the mathematical program Wolfram Mathematica 7.0. 18.43403. (17).

2.2.1. The SIR model without vital dynamics

The SIR model without vital dynamics (18,19) for mastitis transmission in the dairy cow population is a simple mathematical model. The observed population at risk was divided into three groups: the susceptible or cows under risk *(S)*, the cows with clinical mastitis *(I)*, and the recovered cows *(R)*. Related to the nature of the practical problem that should be solved, the symbols *S*, *I* and *R*, which are used to mark the groups according to the SIR model, will be changed. The susceptible group will be denoted with S_c, the cows that suffer from clinical mastitis will be denoted with CM_c, and the recovered cows will be denoted with H_c. Figure 1 shows the SIR model.

$$\boxed{S_c} \xrightarrow{\alpha} \boxed{CM_c} \xrightarrow{\beta} \boxed{H_c}$$

Figure 1. The SIR model without vital dynamics

The fixed size of the total population N is $N = S_c(t) + CM_c(t) + H_c(t)$ for all time t. The functions $S_c(t), CM_c(t)$ and $H_c(t)$ depend on the time t. The rate of infection with which infect the susceptible' cows is denoted by α and the rate of recovery is denoted by β. The time of recovery is $\tau = \frac{1}{\beta}$. This model is described by the following ordinary differential equations:

$$\frac{dS_c}{dt} = -\frac{\alpha S_c CM_c}{N}$$
$$\frac{dCM_c}{dt} = \frac{\alpha S_c CM_c}{N} - \beta CM_c \qquad [1]$$
$$\frac{dH_c}{dt} = \beta CM_c$$

with the initial values $S_c(0) = S_{c_o} > 0,\ CM_c(0) = CM_{c_o} > 0, H_c(0) = H_{c_o} \ge 0$.

By replacement

$$S(t) = \frac{S_c}{N},\ CM(t) = \frac{CM_c(t)}{N}, H(t) = \frac{H_c(t)}{N},$$

this system

$$\frac{dS}{dt} = -\alpha\, S\, CM$$
$$\frac{dCM}{dt} = \alpha\, S\, CM - \beta\, CM \qquad [2]$$
$$\frac{dH}{dt} = \beta\, CM$$

with the initial values $S(0) = S_0 > 0,\ CM(0) = CM_0 > 0, H(0) = H_0 \ge 0$ and $S(t) + CM(t) + H(t) = 1$ for all time t is transformed.

The initial behavior of the system is that we have a small number of infections and no recovered individuals, while most of the population is susceptible. Therefore, we can take $S_{c_o} \approx N$ i.e. $S_0 \approx 1$ then the first and second equations of the system (1] have transformed them to

$$\frac{dS}{dt} \approx -\alpha\, CM$$
$$\frac{dCM}{dt} \approx \alpha\, CM - \beta\, CM = (\alpha - \beta) CM$$

Clear that, if $\alpha > \beta$ i.e. $\frac{\alpha}{\beta} > 1$ then $\frac{dCM}{dt} > 0$ and thus that I is increasing. If $\alpha < \beta$ i.e. $\frac{\alpha}{\beta} < 1$ than $\frac{dCM}{dt} < 0$ thus that CM is lowering i.e. we have a system within the outbreak's initial epidemical stage. Therefore, the quotient $\frac{\alpha}{\beta}$ is an important number for this model and represents the basic reproductive number $\Re_0 = \frac{\alpha}{\beta}$. By definition, the basic reproduction number $\Re_0$ is the average number of secondary infections that a single infectious individual will give rise to for his infection, in the susceptible population.

Clear that, the epidemic will occur if the proportion of invectives increases with time i.e. $\frac{dCM}{dt} > 0$. But we won't have an epidemic for $\frac{dCM}{dt} < 0$.

We can say:

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If $\Re_0 > 1$ then $\frac{dCM}{dt} > 0$ and we will have an epidemic. If $\Re_0 < 1$ then $\frac{dCM}{dt} < 0$ and we won't have an epidemic.
The second equation of the model (2)

$$\frac{dCM}{dt} = \alpha\, S\, CM - \beta\, CM = CM\,(\alpha\, S - \beta)$$

gave us new information that $\frac{dCM}{dt} > 0$, if it is satisfied $\alpha\, S - \beta > 0 \Rightarrow S > \frac{\beta}{\alpha}$. This relation means that the infection will invade in the populations if the condition $S_0 > \frac{\beta}{\alpha}$ i.e. $S_0 > \frac{1}{\Re_0}$ is satisfied. Biologically, this means that the infection will enter the population if the initial number of susceptible individuals $S_0 > \frac{1}{\Re_0}$.

We are not concerned about what will happen to the infection over time. For that purpose, we divide the first equation by the third equation of the model (2) and the following differential equation

$$\frac{dS}{dH} = \frac{-\alpha\, S}{\beta} \Rightarrow \frac{dS}{S} = -\Re_0\, dH\,.$$

By its solving, the solution

$$S(t) = S_0\, e^{-\Re_0 (H(t) - H_0)} \qquad (3)$$

is obtained.
Clear that $H(t) \le 1$ for all time t and the solution $S(t) > 0$ for all time t. Because $\frac{dS}{dt} < 0$, the number of individuals in the susceptible group decrement over the time t. The function $S(t)$ is positive and monotonous function means $\lim_{t\to\infty} S(t) = S_\infty$ (S_∞ is the final size of individuals in the susceptible group). Because $\frac{dH}{dt} > 0$, the number of individuals in the recovery group increment over the time t. The function $R(t)$ is monotonous and bounded by 1 means $\lim_{t\to\infty} H(t) = H_\infty$ (R_∞ is the final size of individuals in the recovery group). The epidemic will be stopped, if the final size of individuals in the infected group $H_\infty = 0$ ($\lim_{t\to\infty} H(t) = H_\infty$).
Because $S(t) + CM(t) + H(t) = 1$, the final sizes and the initial values $H_\infty = 1 - S_\infty, H_0 = 1 - S_0$ are obtained. The solution (3) will have the following form

$$S_\infty = S_0\, e^{\Re_0 (S_\infty - S_0)}\,.$$

This equation can be solved only numerically, and its solution will give us the final size of individuals in the susceptible group.

2.2.2. SIRS-model without vital dynamics

The SIRS model without vital dynamics (20) for the spreading of infectious diseases is a simple mathematical model obtained from the SIR model without vital dynamics. In this model, all recovered individuals return unprotected to the susceptible group, and they can be infected again. In Figure 2, the SIRS model is shown.

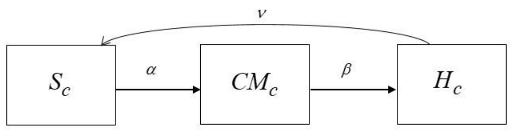

Figure 2. The model SIRS without vital dynamics

The rate by which recovered cows return to the susceptible group is denoted by ν. The time of recovery (in the case of mastitis unspecific immune protection against mastitis pathogens) is $\iota = \frac{1}{\nu}$.
This model is described by the following ordinary differential equations:

$$\frac{dS_c}{dt} = -\frac{\alpha\, S_c\, CM_c}{N} + \nu\, H_c$$

$$\frac{dCM_c}{dt} = \frac{\alpha\, S_c\, CM_c}{N} - \beta\, CM_c$$

$$\frac{dH_c}{dt} = \beta\, CM_c - \nu\, H_c$$

with the initial values $S_c(0) = S_{c_o} > 0,\ CM_c(0) = CM_{c_o} > 0, H_c(0) = H_{c_o} \ge 0$.

We need to observe the long-term behavior of the system, i.e., we are interested in finding equilibrium points where none of the variables change. For the variables that are not changed, their first derivatives must be zero:

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$$-\frac{\alpha S_c CM_c}{N} + \nu H_c = 0$$
$$\frac{\alpha S_c CM_c}{N} - \beta CM_c = 0$$
$$\beta CM_c - \nu H_c = 0$$

By solving this system, two solutions are obtained. This means that model has two equilibrium points: the disease-free equilibrium point (DFE) $(S_c{}^0, CM_c{}^0, H_c{}^0) = (N, 0, 0)$ and the endemic equilibrium point EE $(S_c{}^*, CM_c{}^*, H_c{}^*) = (\frac{N}{\Re_0}, \frac{N}{\Re_0} \cdot \frac{\nu}{\nu+\beta}(\Re_0 - 1), \frac{N}{\Re_0} \cdot \frac{\beta}{\nu+\beta}(\Re_0 - 1))$,

where the basic reproductive number is $\Re_0 = \frac{\alpha}{\beta}$. If $\Re_0 > 1$ then we will have an epidemic. If $\Re_0 < 1$ then we won't have an epidemic. If $\Re_0 < 1$ then the DFE will be a stable node. If $\Re_0 > 1$ then the DFE will be a saddle point (unstable).

If $[\nu(\alpha+\nu)]^2 - \nu(\alpha-\beta)[2(\beta+\nu)]^2 \geq 0$ and $\Re_0 < 1$ then the EE will be a saddle point (unstable).

If $[\nu(\alpha+\nu)]^2 - \nu(\alpha-\beta)[2(\beta+\nu)]^2 \geq 0$ and $\Re_0 > 1$ then the EE will be a stable node. If $[\nu(\alpha+\nu)]^2 - \nu(\alpha-\beta)[2(\beta+\nu)]^2 < 0$ and $\Re_0 > 1$ then the EE will be a stable focus and the following condition $\Re_0 > \frac{[\nu(\alpha+\nu)]^2}{4\beta\nu(\beta+\nu)^2} + 1$.

2.3. Machine learning

For data prediction, Weka software (21) was used. Weka is a collection of machine learning (ML) algorithms for data mining tasks. Weka is widely used in academia, research, and industry for educational purposes, prototyping machine learning solutions, and exploring data mining techniques. For prediction, we used all available data for 1031 cows from the database and classification rules, such as DecisionTable, M5Rules and ZeroR rules. We used cross-validation folds 10.

3. RESULTS

The annual prevalence of clinical mastitis, calculated as the rate per 100 cow/years at risk, per 100 lactation and as lactation incidence risk is shown in Table 1.

Table 1. The annual prevalence rate of clinical mastitis in the observed population of dairy cows

Farms	**Prevalence rate per 100 cows/years at risk**	**Prevalence rate per 100 lactations**	**Lactation incidence risk**
A	24.69%	20.10%	25.00%
B	50.70%	51.35%	95.58%
C	25.59%	19.97%	21.49%
Total	34.14%	30.07%	45.86%

The total prevalence of clinical mastitis for the entire observed population of dairy cows, calculated per 100 cows/years at risk, was 34.14% and 30.07% calculated per 100 lactations. The lactation incidence risk for clinical mastitis was 45.86%.

Table 2 shows the number of cases of clinical mastitis during lactation and the occurrence of recurrent cases during the same lactation.

Table 2. Reoccurrence of clinical mastitis cases within a single lactation

	Cows in lactation that experienced					
Farms	1 case of CM	2 cases of CM	3 cases of CM	4 cases of CM	5 cases of CM	6 cases of CM
A	82.92 %	9.75 %	7.31 %			
B	50.23 %	27.27 %	12.44 %	7.17 %	1.91 %	0.95 %
C	92.36 %	7.63 %				
Total	68.24 %	18.63 %	7.61 %	3.93 %	1.04 %	0.52 %

From the analysis of showed results in Table 2 it might be noticed that most of the cows suffered from one case of clinical mastitis during the same lactation, and the number of cows suffered from recurrent consecutive cases (two, three, four or more) was rare.

The SIR model

The total population of the cows in the trial is $N = 1031$ with the initial values $S_{c_O} = 439,\ CM_{c_O} = 352,\ H_{c_O} = 240$. The rate of infection is $\alpha = 0,3414$ and the rate of recovery is $\beta = 0,6824$. The time of recovery is $\tau = \frac{1}{\beta} = \frac{1}{0,6824} = 1{,}46542$ days plus a nine-day interval needed for the pathogenesis of processes in the mammary gland related to mastitis. The basic reproduction number $\Re_0 = \frac{\alpha}{\beta} = \frac{0,3414}{0,6824} \approx 0,5003 < 1$ i.e. the epidemic will be avoided. In addition to this the fact that i.e. $S_0 = \frac{S_{c_O}}{N} = \frac{439}{1031} \approx 0,4258 > \frac{1}{\Re_0} = \frac{1}{0,5003} = 1{,}9988$.

In Figure 3 is shown the functions $S_c(t),\ CM_c(t)$ and $H_c(t)$:

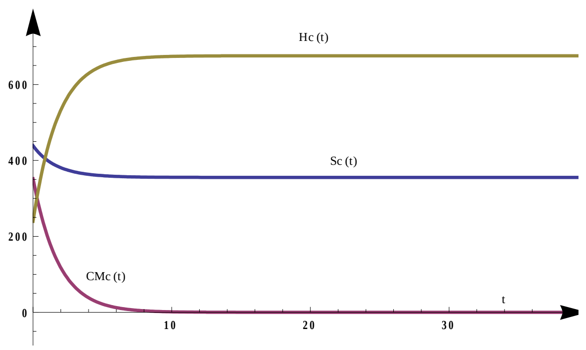
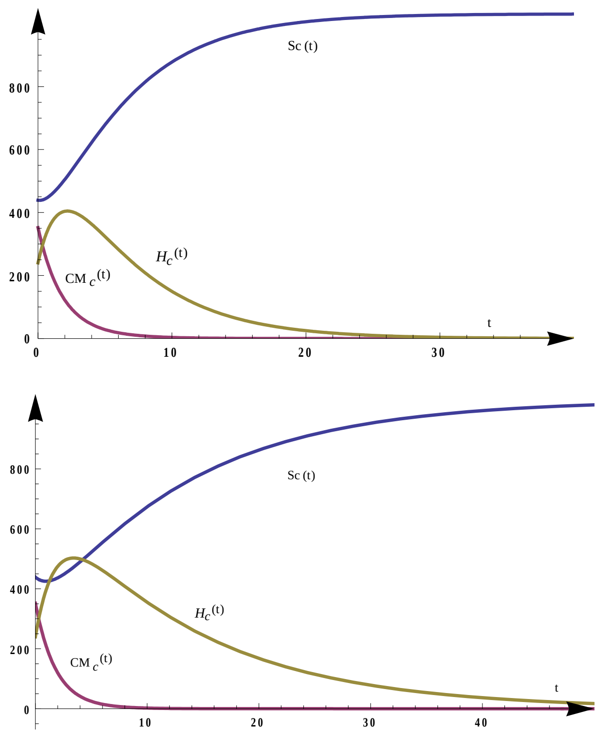


Figure 3. The functions $S_c(t),\ CM_c(t)$ and $H_c(t)$

The SIRS model

The total population of the cows included in the trial is $N = 1031$ with the initial values $S_{c_O} = 439,\ I_{c_O} = 352,\ R_{c_O} = 240$. The rate of infection is $\alpha = 0,3414$ and the rate of recovery is $\beta = 0,6824.$. The basic reproduction number is $\Re_0 = \frac{\alpha}{\beta} = \frac{0,3414}{0,6824} \approx 0,5003 < 1$. The equilibrium point DFE is $DFE = (1031, 0, 0)$.

In our case, we will compare the epidemic behaviour to the recurrence of the disease one time, two, three, four, and five times. In all cases, the disease-free equilibrium points $DFE = (1031, 0, 0)$ and the basic reproduction number $\Re_0 \approx 0,5003 < 1$ are the same. The graphical presentations of the functions $S_c(t),\ CM_c(t)$ and $H_c(t)$ for the different values of the rate by which recovered cows return to the susceptible group at times of recurrence of the disease ν are obtained in Figure 4:



a) $\nu = 0.1863$ **b)** $\nu = 0.0761$

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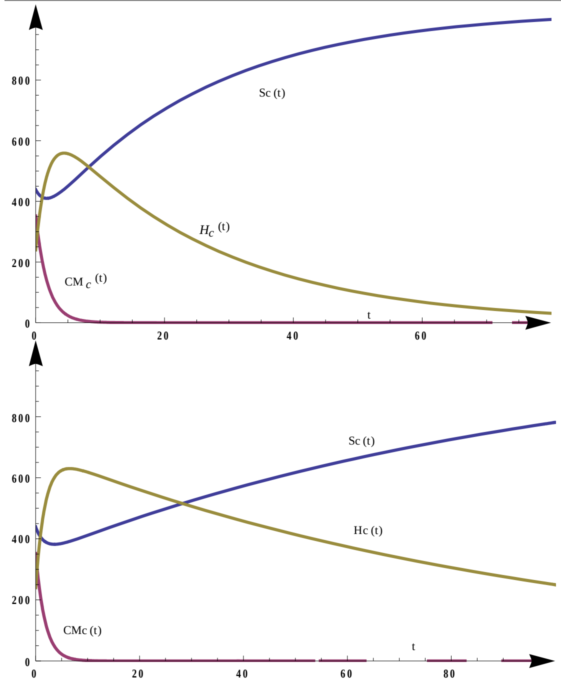
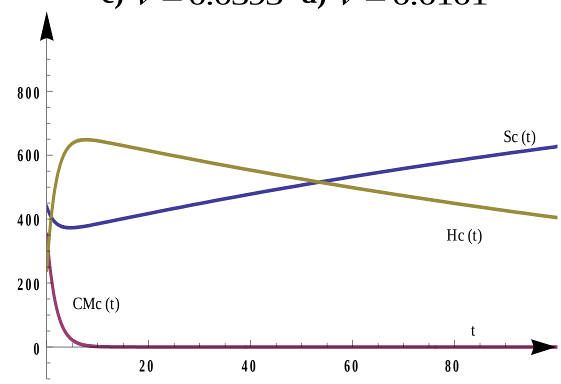


c) $\nu = 0.0393$ d) $\nu = 0.0101$



e) $\nu = 0.0052$

Figure 4. The graphical presentations of the functions $S_c(t)$, $CM_c(t)$ and $H_c(t)$ for the different values of ν

The time of recovery (in case of mastitis unspecific immune protection against mastitis pathogens) $\iota = \frac{1}{\nu}$ for different values of ν (days) are:

- $\iota \approx 5.37$ for $\nu = 0.1863$;
- $\iota \approx 13.14$ for $\nu = 0.0761$;
- $\iota \approx 25.44$ for $\nu = 0.0393$;
- $\iota \approx 99.01$ for $\nu = 0.0101$;
- $\iota \approx 192.31$ for $\nu = 0.0052$.

The disease-free equilibrium point $DFE = (1031, 0, 0)$ is a stable node for all cases of ν.

Results from ML are given in Table 3 below. According to the results, we can see that the best correlation coefficient we have while using decision table rules. That means the strength of a linear relationship between variables is good. With ZeroR we have a negative coefficient. Root mean squared error is small in all cases, but the relative absolute error is smaller while using the decision table and M5Rules. The best results are obtained using a decision table, and the worst are obtained using ZeroR classification rules. So, that means that with the decision table, we can best predict mastitis in dairy herds and with that can help prevent disease.

Table 3. Machine learning classification rules

	Classification rules		
	Decision Table:	M5Rules:	ZeroR:
Correlation coefficient	0.9988	0.8385	-0.0586
Mean absolute error	0.001	0.0109	0.406
Root mean squared error	0.0223	0.3023	0.4506
Relative absolute error	0.2418%	2.6937%	100 %
Root relative squared error	4.9541%	67.0884%	100 %

4. DISCUSSION

The widespread occurrence of clinical mastitis significantly impacts the economic stability of dairy farms due to its high prevalence and associated risks. Mastitis exemplifies a multifactorial disease, suggesting that variations in its prevalence across farms can be attributed to differences in farm management, environmental conditions, breeding systems, hygiene practices, health management, milk production, and the genetic diversity among cows, particularly in their resistance to mastitis.
The SIR model revealed that after a short time, the epidemic will disappear. From the explanation and the graphical presentations, it can be concluded that the stable point *DFE* attracts the trajectories of the system. Biologically speaking, the disease on the

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farms is calming down, and with these parameters of the model, an epidemic cannot occur.

The SIR and SIRS models are types of compartmental models used in epidemiology to describe how diseases spread through populations. These models can also be adapted to predict the occurrence of clinical mastitis in dairy herds by considering the unique aspects of the disease and the way it spreads among cattle. The rate at which the disease spreads depends on the contact rate between susceptible and infected cows and the effectiveness of transmission per contact. The rate of recovery represents the time needed for mastitic cows to return to health (either through natural recovery or treatment). To effectively use SIR or SIRS models for predicting clinical mastitis, data for the number of susceptible, infected, and recovered cows are needed, along with transmission rates of mastitis in the herd, recovery rates and rates at which recovered cows become susceptible again. In the early stages, the number of infected cows increases rapidly due to the high transmission rate. As more cows become infected, the number of susceptible cows declines. The model predicts a peak in infections after a certain period, depending on the transmission and recovery rates. This peak represents the maximum number of cows infected during the outbreak. As infected cows recover or are removed from the population (due to culling or isolation), the number of infected individuals decreases. This decline is influenced by the recovery rate. After a certain period, the SIR model reaches an endemic equilibrium where the number of susceptible, infected, and recovered cows stabilizes. This equilibrium level depends on the balance between transmission and recovery rates. Unlike the SIR model, the SIRS model accounts for the possibility of recovered cows becoming susceptible again. This introduces cyclic behaviour into the model, where individuals move between susceptible, infected, recovered, and susceptible states over time. Due to the cyclic nature of the SIRS model, there can be observed oscillations in the number of infected cows over time. These oscillations represent periodic outbreaks of clinical mastitis as recovered cows become susceptible again, leading to new infections. The SIRS model predicts long-term fluctuations in the prevalence of clinical mastitis within the dairy herd. These fluctuations are influenced by the balance between transmission, recovery, and the rate of susceptibility. Understanding the cyclic behaviour of the SIRS model can inform control strategies. For instance, good management practices aimed at reducing transmission can help dampen the oscillations and stabilize the prevalence of clinical mastitis in the long term.

Therefore, these models can be made more complex and precise by including additional factors like varying susceptibility due to genetic factors, age, or stage of lactation, and by considering external factors like farm management practices.

With the use of the decision table as one of the most used classification rules and cross-validation folds 10 we can best predict mastitis. The decision table classifier scans through the decision table to find precise matches, focusing solely on the features specified in the schema. The cross-validation folds 10 approach involves partitioning a single dataset into ten randomly selected subsets. Nine of these subsets are utilized for training purposes, while one subset is reserved exclusively for testing. This process is iterated ten times, with each iteration selecting a different subset for testing while the remaining nine are used for training.

Single or deep ML models have traditionally been employed to classify cows into healthy and at risk of mastitis, whether clinical or subclinical. Common approaches include decision trees, distance-based methods like support vector machines, clustering models such as k-nearest neighbours and linear discriminant analysis, neural networks, and generalized linear models like logistic regression (20,21,22,23). 22,23,24,25

Recently, there has been a surge in interest in utilizing data collected from both automatic milking recording systems and routine milk recording procedures. These data, easily accessible to farmers and abundant in quantity, can be leveraged for training ML models geared towards classification or regression, aiding in the assessment of traits that may be challenging to measure directly. ML models tailored for dairy cattle have proven effective in early mastitis risk identification (26,23,27,25,28).

5.CONCLUSSIONS

Both the SIR and SIRS models provide valuable insights into the dynamics of clinical mastitis in dairy herds. While the SIR model offers a simplified representation of disease transmission and recovery, the SIRS model accounts for the loss of immunity over time, leading to cyclic behaviour in the prevalence of mastitis. By analyzing the results from these models, veterinarians and farm managers can

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develop effective control strategies to minimize the impact of clinical mastitis on dairy production. With the use of the decision table as one of the most used classification rules and cross-validation folds 10 we can best predict clinical mastitis occurrence in dairy herds. Implementation of a good mastitis prevention program in dairy herds by increasing the rates of control parameters will reduce the mastitis pathogens transmission rates leading to a reduction of mastitis incidence.

CONFLICTS OF INTEREST

The authors state that there are no conflicts of interest concerning the publication of this article.

REFERENCES

1. *Nakov D., Trajchev M. Udder quarter risk factors associated with prevalence of bovine clinical mastitis. Mac Vet Rev. 2012, 35(2):55-64.*
2. *Fetai V., Nakov D., Jashari B., Trajchev M. Economics of milk yield losses in one dairy farm in Macedonia associated with clinical mastitis. Universi-International Journal of Education, Science, Technology, Innovation, Health and Environment 2015, 1(4):104-13.*
3. *Bradley A.J., Leach K.A., Breen J.E., Green L.E., Green M.J. Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales. Veterinary Record 2007, 160(8):253-8.*
4. *Santman-Berends I.M., Lam T.J., Keurentjes J., Van Schaik G. An estimation of the clinical mastitis incidence per 100 cows per year based on routinely collected herd data. Journal of dairy science 2015, 98(10):6965-77.*
5. *Tommasoni C., Fiore E., Lisuzzo A., Gianesella M. Mastitis in dairy cattle: On-farm diagnostics and future perspectives. Animals 2023, 13(15):2538.*
6. *Hagnestam C., Emanuelson U., Berglund B. Yield losses associated with clinical mastitis occurring in different weeks of lactation. Journal of dairy science 2007, 90(5):2260-70.*
7. *Nakov D., Trajchev M. Monitoring udder health and milk hygiene on-farm using quick screening methods. Journal of Agriculture and Plant Sciences 2018, 16(2):57-63.*
8. *Nyman A.K., Ekman T., Emanuelson U., Gustafsson A.H., Holtenius K., Waller K.P., Sandgren C.H. Risk factors associated with the incidence of veterinary-treated clinical mastitis in Swedish dairy herds with a high milk yield and a low prevalence of subclinical mastitis. Preventive veterinary medicine 2007, 78(2):142-60.*
9. *Valde J.P., Lystad M.L., Simensen E., Østerås O. Comparison of feeding management and body condition of dairy cows in herds with low and high mastitis rates. Journal of dairy science 2007, 90(9):4317-24.*
10. *Nakov D., Hristov S., Andonov S., Trajchev M. Udder-related risk factors for clinical mastitis in dairy cows. Veterinarski arhiv 2014, 84(2):111-227.*
11. *Rowe S., Tranter W., Laven R. Longitudinal study of herd udder hygiene and its association with clinical mastitis in pasture-based dairy cows. Journal of Dairy Science 2021, 104(5):6051-60.*
12. *Levison L.J., Miller-Cushon E.K., Tucker A.L., Bergeron R., Leslie K.E., Barkema H.W., DeVries T.J. Incidence rate of pathogen-specific clinical mastitis on conventional and organic Canadian dairy farms. Journal of dairy science 2016, 99(2):1341-50.*
13. *Trajchev M., Nakov D., Petrovska M., Jankoska G. Mastitis pathogens and their antimicrobial susceptibility in early lactating dairy cows. The Journal Agriculture and Forestry 2017, 63(1).*
14. *Abdi R.D., Gillespie B.E., Ivey S., Pighetti G.M., Almeida R.A., Kerro Dego O. Antimicrobial resistance of major bacterial pathogens from dairy cows with high somatic cell count and clinical mastitis. Animals 2021, 11(1):131.*
15. *Izneid B.A., Gurmu E.D., Obsu L.L., Melese A.S., Kanan M., Al-Qerem A. Optimal Control Strategy on Mathematical Model for the Dynamics of Mastitis. Appl. Math. 2023, 17(4):699-711.*
16. *Sviland S., Waage S. Clinical bovine mastitis in Norway. Preventive Veterinary Medicine 2002, 54(1):65-78.*
17. *Wolfram Mathematica 7.0. 18.43403 Informer Technologies, Inc. https://wolfram-mathematica.informer.com/7.0/ accessed 29/4/2024*
18. *Philipps S, Rossi D, Von Arb R, Capaldi A. Mathematical models of infectious diseases: Two-strain infections in metapopulations. 2011.*
19. *Doeschl-Wilson A. Modelling Epidemics. Lecture 5: Deterministic compartmental epidemiological models in homogeneous populations. https://jvanderw.une.edu.au/L5_ModellingEpidemics1.pdf accessed 29/4/2024.*
20. *Dadlani A., Afolabi R.O., Jung H., Sohraby K., Kim K. Deterministic models in epidemiology: from modelling to implementation. arXiv preprint arXiv:2004.04675. 2020.*
21. *WEKA Unveils Powerful AI-Native Data Platform Appliance for NVIDIA DGX SuperPOD with NVIDIA DGX H100 Systems. https://www.weka.io/ accessed 29/4/2024.*

22. *Mammadova N.M., Keskin I. Application of neural network and adaptive neuro-fuzzy inference system to predict subclinical mastitis in dairy cattle. Indian Journal of Animal Research 2015, 49(5):671-9.*
23. *Sitkowska B., Piwczynski D., Aerts J., Kolenda M., Özkaya S. Detection of high levels of somatic cells in milk on farms equipped with an automatic milking system by decision trees technique. Turkish Journal of Veterinary & Animal Sciences 2017, 41(4):532-40.*
24. *Ebrahimi M., Mohammadi-Dehcheshmeh M., Ebrahimie E., Petrovski K.R. Comprehensive analysis of machine learning models for prediction of sub-clinical mastitis: Deep Learning and Gradient-Boosted Trees outperform other models. Computers in biology and medicine 2019, 114, 103456.*
25. *Bobbo T., Biffani S., Taccioli C., Penasa M., Cassandro M. Comparison of machine learning methods to predict udder health status based on somatic cell counts in dairy cows. Scientific Reports 2021, 11(1):13642.*
26. *Fadul-Pacheco L., Delgado H., Cabrera V.E. Exploring machine learning algorithms for early prediction of clinical mastitis. International Dairy Journal 2021, 119:105051.*
27. *Ebrahimie E., Ebrahimi F., Ebrahimi M., Tomlinson S., Petrovski K.R. Hierarchical pattern recognition in milking parameters predicts mastitis prevalence. Computers and electronics in agriculture 2018, 147:6-11.*
28. *Tian H., Zhou X., Wang H., Xu C., Zhao Z., Xu W., Deng Z. The Prediction of Clinical Mastitis in Dairy Cows Based on Milk Yield, Rumination Time, and Milk Electrical Conductivity Using Machine Learning Algorithms. Animals 2024, 14(3):427.*

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Review
Pregledni rad

ZNAČAJ I METODE PROCENE INTRAINDIVIDUALNE VARIJABILNOSTI KRVNIH PARAMETARA U VETERINARSKOJ MEDICINI

THE SIGNIFICANCE AND METHODS OF ASSESSMENT OF BLOOD PARAMETER VARIABILITY IN VETERINARY MEDICINE

Sandra Nikolić[1], Annamaria Galfi Vukomanović[1], Mira Majkić[1], Jovan Spasojević[1], Ivan Galić[1], Tijana Kukurić[1]

[1]Univerzitet u Novom Sadu, Poljoprivredni fakultet, Departman za veterinarsku medicinu Novi Sad
Korespodentni autor: Sandra Nikolić, neckovs021@gmail.com

S.N. https://orcid.org/0000-0001-7337-9937, A.G.V. https://orcid.org/0000-0002-5361-1070, M.M. https://orcid.org/0009-0007-0110-7327, J.S. https://orcid.org/0000-0001-5654-5796, I.G. https://orcid.org/0000-0002-6281-0776, T.K. https://orcid.org/0000-0003-2561-8555

SAŽETAK

Intraindividualne varijacije su promene koje se uočavaju unutar jedne jedinke prilikom ponovne procene u nekom vremenskom intervalu i određene su intraindividualnim koeficijentom varijacije (CVi), koji pokazuje stepen varijabilnosti rezultata iz uzorka u odnosu na aritmetičku sredinu populacije. Viši CVi ukazuje na veću varijabilnost unutar jedinke, odnosno manju stabilnost ispitivanog parametra. Izračunavanjem CVi iz ponovljenih merenja, može se proceniti koliko izmereni parametri fluktuiraju kod pojedinačne ispitivane životinje tokom vremena. Odnos individualne i grupne biološke varijacije predstavlja indeks individualnosti (IoI) za ispitivani analit. Ovaj indeks određuje da li je za određeni analit prikladnije koristiti referentne intervale zasnovane na populaciji ili referentne intervale zasnovane na subjektu, odnosno predviđa korisnost referentnih intervala. Ovaj pokazatelj varijacije pruža smernice da će ukoliko je intraindividualna biološka varijacija veća u odnosu na interindividualnu varijaciju, (indeks individualnosti je $\geq$ 1,4), referentni intervali zasnovani na populaciji biti od najveće koristi za sve jedinke, dok ukoliko je intraindividualna biološka varijacija manja u odnosu na interindividualnu varijaciju (indeks individualnosti je $\leq$ 0,6) predlaže se korišćenje RCV vrednosti (*reference change value*, RCV). Izračunavanje RCV vrednosti odnosno kritične razlike je metoda koja se koristi za utvrđivanje da li je razlika u dve uzastopne analize značajna i biološki relevantna, prilikom čega se uzimaju u obzir i faktori povezani sa aktivnošću u laboratoriji (preanalitička i analitička greška), kao i faktori povezani sa normalnom intraindividualnom biološkom varijacijom. Na kraju, utvrđivanje neophodnog broja uzoraka za procenu homeostatskih okvira je još jedan metod za procenu intraindividualne varijabilnosti. Razumevanje intraindividualne varijabilnosti krvnih parametara u veterinarskoj medicini je od krucijalnog značaja za postavljanje tačne dijagnoze i monitoring zdravstvenog stanja životinja. Na osnovu upotrebe metoda intraindividualne varijabilnosti mogu se detektovati diskretne promene tokom vremena koje mogu ukazivati na progresiju ili regresiju oboljenja, a može se pratiti i odgovor na terapiju i po potrebi izvršiti njena korekcija.

Ključne reči: intraindividualna varijabilnost, koeficijent varijabilnosti (CVi), kritična razlika (RCV), indeks individualnosti (IoI), neophodan broj uzoraka za procenu homeostatskih okvira (NHSP)

ABSTRACT

Changes within an individual during re-evaluation within a certain period are referred to as intraindividual variations, and they are determined by the intraindividual coefficient of variation (CVi), which indicates the degree of variability of the sample results in comparison to the population's arithmetic mean. A higher CVi denotes less stability of the investigated parameter and greater variability within the individual. By calculating CVi from repeated measurements, one can estimate how much the measured parameters fluctuate in an individual over time. The ratio of individual and group biological variation represents the index of individuality (IoI) for the examined analyte. This index determines whether it is more appropriate to use population-based reference intervals or subject-based reference intervals for a particular analyte, i.e. predicts the utility of the reference intervals. It also suggests that if intraindividual biological variation is greater than interindividual variation, (individuality index is ≥ 1.4), population-based reference intervals will be most useful for all individuals. If intraindividual biological variation is less than the interindividual variation (individuality index is ≤ 0.6), then the RCV value (reference change value, or RCV) should be used. Calculating the RCV value is a method used to determine whether the difference in two consecutive analyses is significant and biologically relevant, taking into account factors related to laboratory activity (preanalytical and analytical error), as well as factors related to normal intraindividual biological variation. Finally, determining the number of samples required for assessing the homeostatic setting point is another method used to evaluate intraindividual variability. In veterinary medicine, it is essential to understand the intraindividual variability of blood parameters to accurately diagnose patients and monitor their health. Subtle changes over time that may suggest a progression or regression of the disease can be detected by the use of intraindividual variability methods. Additionally, the response to treatment can be monitored and, if necessary, adjusted.

Key words: intra-individual variability, coefficient of variability (CVi), critical difference (RCV), index of individuality (IoI), number of samples for homeostatic setting point evaluation (NHSP)

DEFINICIJA REFERENTNIH INTERVALA

Laboratorijska analiza krvi predstavlja metod koji se najčešće upotrebljava kako u humanoj, tako i u veterinarskoj medicini prvenstveno u cilju detekcije oboljenja i usmeravanju terapije kod pacijenata, a zatim i u svrhe preventivne provere zdravstvenog statusa jedinke, kako bi se na vreme otkrio ili prevenirao nastanak oboljenja.

U cilju donošenja kliničke odluke, široko je rasprostranjena upotreba referentnih intervala krvnih parametara. Oni predstavljaju sastavnu komponentu dijagnostičkog procesa u laboratorijama (1) i najčešće se koriste kako bi se procenili ili poredili rezultati krvi dobijeni od obolelih jedinki, a takođe i kao osnov za postavljanje dijagnoze oboljenja. Osim ovog aspekta, utvrđivanje vrednosti laboratorijskih krvnih parametara može takođe da pruži uvid u metaboličke i fiziološke procese koji se odvijaju u organizmu.

Referentni intervali predstavljaju intervale izračunate na osnovu grupe ili populacije zdravih jedinki iste vrste za određeno ispitivanje, a nazivaju se još i populacijskim referentnim intervalima (population-based reference intervals) (2). Takođe se mogu definisati i kao intervali kojima je obuhvaćena većina jedinki iz date populacije sa karakteristikama sličnim referentnoj grupi, a isključene jedinke čije se karakteristike razlikuju od referentne grupe (3). Referentni intervali imaju svoju donju i gornju granicu koja obuhvata središnjih 95% referentnog intervala izmerenih vrednosti, dobijenih iz uzoraka zdrave referentne grupe (4,5,6). Ostalih 5% jedinki biva isključeno iz grupe referentnih zdravih jedinki, jer odstupa od utvrđenih referentnih vrednosti (konvencionalno 2,5% vrednosti se nalaze ispod i 2,5% vrednosti iznad granica referentnog intervala), a takav nalaz će biti označen kao abnormalan (3).

Iako je upotreba referentnih vednosti utvrđenih na osnovu populacije univerzalno prihvaćena, često je problematičan sam metod utvrđivanja ovih vrednosti, pa je ova tema ne retko predmet diskusije u literature (1). Naime, dobro je poznato da postoje rase čije vrednosti hematoloških i biohemijskih parametara variraju u odnosu na referentne intervale u smislu da se fiziološki nalaze iznad ili ispod granične vrednosti referentnog opsega za populaciju. Takođe, ova pojava može biti vezana i za uzrast, pol i druge karakteristike jedinke. U ovim slučajevima može se desiti da rezultati određenog testa ovakvih jedinki budu proglašeni patološkim, a da su objektivno samo posledica biološke varijacije. Istraživanje uzroka varijacije parametara je uvek preporučljivo, kako bi se isključile preanalitičke i analitičke smetnje i fiziološki izvori varijacije koji utiču na verodostojnost samog nalaza.

Uzimajući u obzir navedeno, poslednjih godina, u humanoj medicini, uveliko se koristi, a u veterinarskoj medicini počinje da se istražuje, koncept referentnih vrednosti baziranih na subjektu (subject-based reference values), što podrazumeva ponovljeno uzorkovanje od iste životinje. Ovakve referentne vrednosti, izračunate iz podataka o biološkoj varijaciji, mogu se koristiti za utvrđivanje

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da li se razlike u dva uzastopna merenja mogu pripisati prirodnoj varijabilnosti, preanalitičkim i analitičkim greškama, ili nekim drugim uzrocima ove razlike kao što su uticaj bolesti ili terapije.

BIOLOŠKA VARIJACIJA, INTRAINDIVIDUALNA I INTERINDIVIDUALNA VARIJACIJA I NJENI POKAZATELJI

Biološka varijacija se definiše kao nasumična fluktuacija analita oko osnovne vrednosti za svaku jedinku ili grupu jedinki (7,8), a obuhvata analitičku varijabilnost (Va), intraindividualnu (Vi) i interindividualnu varijabilnost (Vg), čija se mera obično procenjuje na osnovu njihovih koeficijenata varijacije (CV). Koeficijent varijacije kao statistička mera disperzije podataka od aritmetičke sredine pokazuje stepen varijabilnosti rezultata iz uzorka u odnosu na aritmetičku sredinu populacije. Predstavlja se formulom kao odnos standardne devijacije (SD) i aritmetičke sredine (Mean) (9).

CV = 100 × (Standardna devijacija/Aritmetička sredina)

Analitička varijabilnost (Va) odnosno nasumična greška je povezana sa svakim laboratorijskim rezultatom, pri čemu veličina ove greške zavisi od metodološkog pristupa i tipa analizatora. Utvrđuje se tako što se vrše ponovljena merenja iz jednog te istog uzorka i izračunava analitički koeficijent varijacije preko aritmetičke sredine i standardne devijacije. Što je manji koeficijent analitičke varijabilnosti, manja je i nepreciznost rezultata (10). Analitička varijabilnost većine promenljivih je znatno niža u odnosu na inter- i intraindividualnu varijabilnost zbog visokog nivoa kontrole kvaliteta u laboratorijama. Zbog toga se biološka varijacija uglavnom izražava kroz intra- i interindividualnu (8). Intraindividualne varijacije su promene koje se uočavaju unutar jedne jedinke prilikom ponovne procene u nekom vremenskom razmaku ili okolnostima i određene su intraindividualnim koeficijentom varijacije (CVi), dok su interindividualne (grupne) varijacije razlike koje se uočavaju između različitih jedinki i određene su interindividualnim koeficijentom varijacije (CVg) (7,11). Viši CVi ukazuje na veću varijabilnost unutar jedinke, odnosno manju stabilnost izmerenog parametra. Izračunavanjem CVi iz ponovljenih merenja poreklom od iste jedinke, može se proceniti koliko izmereni parametri fluktuiraju kod pojedinačne ispitivane životinje tokom vremena. Nasuprot tome, niži CVi govori u prilog stabilnosti i postojanosti izmerenih parametara tokom određenog vremenskog perioda. Razumevanje CVi vrednosti pomaže pri ustanovljavanju “normalnih” intervala i detekciji abnormalnih varijacija krvnih parametara, utvrđivanju zdravstvenog stanja životinje, praćenju progresije oboljenja i proceni efikasnosti terapije. Takođe, može da ukaže i na frekvenciju kojom bi trebalo vršiti praćenja određenog analita na osnovu njegove utvrđene varijabilnosti unutar jedinke. Pored svega navedenog, neophodno je imati na umu da CVi obezbeđuje vredan uvid u intraindividualnu varijabilnost, ali ne može obuhvatati sve izvore varijabilnosti. Faktori kao što su stres, način ishrane, laktacija, bolest i terapija mogu uticati na krvne parametre i doprinositi varijabilnosti iznad one koja je izmerena samo upotrebom CVi vrednosti.

Sledeći metod procene intraindividualne varijabilnosti ispitivanih krvnih parametara jeste *indeks individualnosti (IoI)*, koji predstavlja odnos individualne i grupne biološke varijacije za ispitivani analit. Ovaj indeks određuje da li je za određeni analit prikladnije koristiti referentne intervale zasnovane na populaciji ili referentne intervale zasnovane na subjektu, odnosno predviđa korisnost referentnih intervala (10,12). Većina analita, kao što je to slučaj sa kalcijumom, holesterolom i kreatininom, imaju izraženu individualnost, odnosno nizak indeks individualnosti, što znači da je stepen biološke varijacije unutar jedinke mnogo manji u odnosu na stepen biološke varijacije unutar populacije. U ovakvim slučajevima populacijski referentni intervali nisu naročito korisni u proceni da li je rezultat testa “normalan” ili ne, te bi verovatno bilo bolje porediti dobijene rezultate sa referentnim intervalima utvrđenim za tu samu jedinku (10). Indeks individualnosti određen analitičkim, intraindividualnim i interindividualnim koeficijentom varijacije pruža smernice da će ukoliko je intraindividualna biološka varijacija veća u odnosu na interindividualnu varijaciju, (indeks individualnosti je $\geq$ 1,4), referentni intervali zasnovani na populaciji biti od najveće koristi za sve jedinke, dok ukoliko je intraindividualna biološka varijacija manja u odnosu na interindividualnu varijaciju (indeks individualnosti je $\leq$ 0,6) treba koristiti referentne intervale zasnovane na subjektu (12,13,14). Takođe, ovo znači da ukoliko je indeks individualnosti nizak, odnosno da je individualnost visoka, varijabilnost vrednosti za individuu zauzima samo mali deo referentnog intervala. Sa druge

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strane, ako je indeks individualnosti visok, odnosno individualnost je niska, disperzija vrednosti kod svake jedinke ponaosob slična je disperziji među jedinkama populacije predstavljene referentnim intervalima (15).
Vrednosti indeksa individualnosti i njihova interpretacija su sledeći:

- <0.6 (nizak indeks individualnosti) - koristiti referentne intervale bazirane na subjektu ili kritičnu grešku (RCV) (2, 7,12);
- 0.6-1.4 (ekvivokalni indeks individualnosti) - koristiti populacijske referentne vrednosti uz oprez. Na žalost, najveći broj rezultata testova potpada u ovu kategoriju koja nije od prevelike pomoći i
- >1.4 (visok indeks individualnosti) - koristiti populacijske referentne vrednosti.

Formula na osnovu koje se uobičajeno izračunavaju indeksi individualnosti može se predstaviti sledećom jednačinom u kojoj se u brojiocu nalaze CVi koji predstavlja koeficijent intraindividualne varijabilnosti i CVa odnosno koeficijent analitičke varijabilnosti, a u imeniocu se nalazi CVg odnosno koeficijent interindividualne varijabilnosti (16).

$$IoI = \frac{\sqrt{CVi^2 + CVa^2}}{\mathrm{CVg}}$$

Kod varijabli kod kojih postoji pretpostavka normalnosti, indeksi individualnosti se mogu računati iz koeficijenata varijacije na osnovu recipročne formule odnosno formule u kojoj se koeficijent interindividualne varijacije (CVg) postavlja kao brojilac i to je tzv. inverzirani indeks individualnosti koji rezultira u višim vrednostima kod porasta intraindividualne varijacije (12,17). Formula inverziranog indeksa individualnosti može se predstaviti na sledeći način:

$$IoI = \frac{\mathrm{CVg}}{\sqrt{CVi^2 + CVa^2}}$$

Pri ovakvom načinu izračunavanja, indeksi individualnosti:
≥1,67 ukazuju na to da su u takvim slučajevima referentni intervali zasnovani na subjektu od veće koristi u odnosu na referentne intervale zasnovane na populaciji,
≤ 0,7 treba koristiti populacijske referentne intervale.
0,7 - 1,67 spadaju u ekvivokalne odnosno u nesigurne, tako da se u ovim slučajevima bazira na populacijske referentne intervale uz oprez.

Uopšteno govoreći, kada je indeks individualnosti po uobičajenoj formuli niži od 0,6, a po inverziranoj formuli viši od 1,67 predlaže se korišćenje RCV vrednosti (*reference change value,* RCV) odnosno kritične razlike koja predstavlja sledeći metod za procenu intraindividualne varijabilnosti krvnih parametara (18).
Biološka varijacija često može da komplikuje interpretaciju kliničkih rezultata, naročito onih dobijenih konsekutivnom analizom uzorka poreklom od iste jedinke. Metoda koja se koristi za utvrđivanje da li je razlika u dve uzastopne analize značajna i biološki relevantna jeste izračunavanje RCV vrednosti (reference change value, RCV) prilikom čega se uzimaju u obzir i faktori povezani sa aktivnošću u laboratoriji (preanalitička i analitička greška), kao i faktori povezani sa normalnom intraindividualnom biološkom varijacijom (18). RCV predstavlja najmanju razliku dva uzastopna merenja koja izdvaja rezultat iz zone nepouzdanosti merenja. Nepouzdanost merenja predstavlja ekspresiju statističke varijanse vrednosti usled izmerenog kvantiteta. Nijedan metod merenja nije u potpunosti precizan kada je merenje kvantiteta u pitanju, jer vrednosti zavise od različitih faktora kao što su sistem merenja, metod merenja, nivo usavršenosti vršioca merenja, kao i okruženja. Čak i ako se količina meri nekoliko puta uzastopno, na isti način i u istoj situaciji, vrednosti merenja će se razlikovati od jednog merenja do drugog (19). RCV definiše procenat promene koji treba da se nadvisi kako bi se utvrdila značajna razlika između dva uzastopna merenja (18). RCV ima svoj opseg vrednosti odnosno donju i gornju granicu izvan kojih se promene smatraju značajnim, a ne samo rezultatom fizioloških varijacija ili analitičke nepreciznosti (11).
Iz svega prethodnog mogli bismo izvući konačnu definiciju RCV, a to je da se najmanje razlike između dve dobijene koncentracije ispitivanog analita koje se mogu povezati sa realnom promenom zdravstvenog stanja pacijenta nazivaju *„reference change values“* (RCV) ili kritične razlike. Procena na osnovu RCV je dosta korisnija u odnosu na populacijske referentne intervale i objektivnija jer uključuje kako biološku (intraindividualnu) tako i analitičku varijaciju, odnosno RCV daje objektivnu prednost interpretaciji laboratorijskog nalaza pacijenta kada je dostupan prethodni nalaz (20). Baza podataka o biološkoj varijaciji se stalno dopunjuje, izračunate RCV vrednosti mogu se naći u literaturi, što je češći slučaj u humanoj medicini u

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odnosu na veterinarsku, a neke strane države uvele su izdavanje podatka o RCV uz referentne intervale kao pomoć pri interpretaciji nalaza (10,21).
RCV vrednosti se računaju prema sledećoj formuli (22):

$$RCV = Z \times \sqrt{2} \times \sqrt{CV_A{}^2 + CV_I{}^2}$$

U ovoj formuli Z predstavlja odgovarajući broj standardnih devijacija zasnovanih na željenoj verovatnoći (za dvosmerne promene 1.96 = 95% i 2.58 = 99%), √2 ukazuje na to da procenjujemo promenu koja se javlja između dva rezultata u seriji, CVa je koeficijent analitičke varijabilnosti i CVi koeficijent intraindividualne varijabilnosti (18,22,23). Kvantitativni podaci o komponentama biološke varijacije se koriste u svrhu utvrđivanja specifikacija kvaliteta laboratorijske analize (24), zatim utvrđivanja neophodnog broja uzoraka kako bi se procenilo homeostatsko okruženje jedinke, kao i u proceni korisnosti populacijskih referentnih intervala (25) i izračunavanja referentne vrednosti promene (RCV) neophodne za procenu značajnosti promena u serijskim rezultatima jedinke (23,26,27). Takođe, referentni intervali zasnovani na subjektu (RCV), koji se izračunavaju na osnovu podataka o biološkoj varijaciji, mogu se koristiti pri utvrđivanju da li je razlika u dva uzastopna merenja rezultat prirodne varijabilnosti ili su prisutni neki drugi uzroci kao što su bolest ili terapija koji dovode do evidentne razlike (7,12,28).
U poslednje vreme, razmatra se mogućnost da će se ukoliko se uobičajeni proračuni za RCV primene na više od dva uzastopna uzorka, povećati verovatnoća dobijanja lažno pozitivnih rezultata (ako promena za rezultat ima porast) kao i lažno negativnih rezultata (ukoliko promena za rezultat ima opadanje), naročito ako je visok ukupni koeficijent varijacije (CVT=30-40%) (29). Kako bi rešili ove probleme različiti autori su razvili nove modele zasnovane na log-normalnoj distribuciji podataka o biološkoj varijaciji uz preporuku da se koriste faktori referentne promene za izračunavanje gornjeg i donjeg limita za značajne promene u sekvenci rezultata (29).
Potencijalni nedostaci upotrebe RCV vrednosti su:

- Velika količina statističkih informacija koja zbunjuje kliničare
- Upotreba Z skora koja eliminiše kliničku procenu
- RCV vrednosti mogu biti zavisne od učestalosti testiranja
- Neke biološke varijacije mogu biti zavisne od zdravstvenog stanja
- Pravilna primena RCV vrednosti zahteva prefinjeni laboratorijski informacioni sistem upravljanja (*Laboratory information management system /LIMS*)
- Neophodna je edukacija laboratorijskog osoblja i kliničara
- Terminologija može biti zbunjujuća (30)

Na kraju, utvrđivanje neophodnog broja uzoraka za procenu homeostaze je još jedan metod za procenu intraindividualne varijabilnosti. Iako je u uslovima zdravlja samo jedan laboratorijski rezultat dovoljan da se procene homeostatski okviri jedinke, preciznija procena homeostatskih okvira se dobija izračunavanjem srednjih vrednosti višestrukih uzoraka (npr. multiplih godišnjih rezultata ispitivanja). Neophodan broj uzoraka za procenu homeostatskih okvira sa zadatom preciznošću se može izračunati na osnovu ocene standardne greške aritmetičke sredine. Formula koja se koristi je sledeća:

$$\mathrm{NHSP} = \left(z \times \frac{\sqrt{\mathrm{CV}_{\mathrm{I}}^2 + \mathrm{CV}_{\mathrm{A}}^2}}{\mathrm{D}} \right)^2$$

U ovoj jednačini Z predstavlja odgovarajući broj standardnih devijacija zasnovanih na željenoj verovatnoći (za dvosmerne promene 1.96 = 95% i 2.58 = 99%), CVa je koeficijent analitičke varijabilnosti, CVi koeficijent intraindividualne varijabilnosti, a D predstavlja dozvoljeni procenat odstupanja od homeostatskih okvira jedinke. Obično se dozvoljava maksimalno do10% odstupanja što je adekvatno za analite sa vrednostima $CVI \geq 6{,}67$, ali previsoko za analite niske individualnosti, npr. elektrolite. Za analite kod kojih je $CVI < 6{,}67$, preporučuje se da vrednost $D = 1{,}5 \times CVI$, do maksimalnog odstupanja od 10%. Istraživanja ukazuju da se homeostatski okviri za većinu analita kod zdravih pasa, mačaka i konja, mogu utvrditi sa verovatnoćom od 95% iz manje od 4 uzorka, dok se kod bolesnih jedinki neophodan broj uzoraka značajno povećava(31).
Razumevanje intraindividualne varijabilnosti krvnih parametara u veterinarskoj medicini je od krucijalnog značaja za postavljanje tačne dijagnoze i monitoring zdravstvenog stanja životinja. Na osnovu upotrebe metoda intraindividualne varijabilnosti mogu se detektovati diskretne promene tokom

vremena koje mogu ukazivati na progresiju ili regresiju oboljenja, a može se pratiti i odgovor na terapiju i po potrebi izvršiti njena korekcija.

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LITERATURA

1. *Friedrichs K.R., Harr K.E., Freeman K.P., Szladovits B., Walton R.M., Barnhart K.F., et al. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Pathol 2012, 41(4):441–53.*
2. *Reference intervals. Eclinpath.com. 2013; Available from: https://eclinpath.com/test-basics/reference-intervals/*
3. *Katayev A., Balciza C., Seccombe D.W. Establishing reference intervals for clinical laboratory test results: is there a better way?: Is there a better way? Am J Clin Pathol 2010, 133(2):180–6.*
4. *Özcürümez M.K., Haeckel R., Gurr E., Streichert T., Sack U. Determination and verification of reference interval limits in clinical chemistry. Recommendations for laboratories on behalf of the Working Group Guide Limits of the DGKL with respect to ISO Standard 15189 and the Guideline of the German Medical Association on Quality Assurance in Medical Laboratory Examinations (Rili-BAEK). Journal of Laboratory Medicine 2019, 43(3):127–33.*
5. *Pak S.I., Han H.J. Evaluation of clinical usefulness of reference interval of some selected hematological parameters in canine blood. Korean J Vet Res 2004, 44(4):649-653.*
6. *Regis M., Postma T.A., van den Heuvel E.R. A note on the calculation of reference change values for two consecutive normally distributed laboratory results. Chemometr Intell Lab Syst 2017, 171:102–11.*
7. *Falkenö U., Hillström A., von Brömssen C., Strage E.M. Biological variation of 20 analytes measured in serum from clinically healthy domestic cats. J Vet Diagn Invest 2016, 28(6):699–704.*
8. *Trumel C., Monzali C., Geffré A., Concordet D.V., Hourqueig L., Braun J.-P.D., et al. Hematologic and biochemical biologic variation in laboratory cats. J Am Assoc Lab Anim Sci 2016, 55(5):503-9.*
9. *Pélabon C., Hilde C.H., Einum S., Gamelon M. On the use of the coefficient of variation to quantify and compare trait variation. Evol Lett. 2020, 4(3):180–8.*
10. *Fierdoz O. Essential laboratory knowledge for the clinician. Laboratory testing forms an integral part of patient management. CME 2012, 30(7):244-8.*
11. *Campora C., Freeman K.P., Baral R. Clinical application of biological variation data to facilitate interpretation of canine and feline laboratory results: Biological variation applied to clinical practice. J Small Anim Pract 2018, 59(1):3–9.*
12. *Baral R.M., Dhand N.K., Freeman K.P., Krockenberger M.B., Govendir M. Biological variation and reference change values of feline plasma biochemistry analytes. J Feline Med Surg 2014, 16(4):317–25.*
13. *Pagitz M., Frommlet F., Schwendenwein I. Evaluation of biological variance of cystatin C in comparison with other endogenous markers of glomerular filtration rate in healthy dogs. J Vet Intern Med 2007, 21(5):936–42.*
14. *Badrick T. Biological variation: Understanding why it is so important? Pract Lab Med 2021, 23(e00199):e00199.*
15. *Braga F., Panteghini M. Generation of data on within-subject biological variation in laboratory medicine: An update. Crit Rev Clin Lab Sci 2016, 53(5):313–25.*
16. *Bourgès-Abella N.H., Gury T.D., Geffré A., Concordet D., Thibault-Duprey K.C., Dauchy A., et al. Reference intervals, intraindividual and interindividual variability, and reference change values for hematologic variables in laboratory beagles. J Am Assoc Lab Anim Sci 2015, 54(1):17–24.*
17. *Ruaux C.G., Carney P.C., Suchodolski J.S., Steiner J.M. Estimates of biological variation in routinely measured biochemical analytes in clinically healthy dogs. Vet Clin Pathol 2012, 41(4):541–7.*
18. *Nunes L.A.S., Brenzikofer R., de Macedo D.V. Reference change values of blood analytes from physically active subjects. Eur J Appl Physiol 2010, 110(1):191–8.*
19. *Hong J., Cho E.-J., Kim H.-K., Lee W., Chun S., Min W.-K.. Application and optimization of reference change values for Delta Checks in clinical laboratory. J Clin Lab Anal 2020, 34(12):e23550.*

ISBN 978-86-7520-611-8

20. *Fernandez D., Avinash S.S., Malathi M., Shivashankara A.R., Kumar A., Fernandez P. Establishing the reference change values (RCVs) and validating the delta check auto-verification in a clinical biochemistry laboratory. Muller j med sci res 2017, 8(1):42.*
21. *Krleza J.L. Laboratorijska medicina: Predanalitička pogreška, interferencije i interpretacija nalaza. Klinika za dječje bolesti Zagreb 2014.*
22. *Fernández-Grande E., Valera-Rodriguez C., Sáenz-Mateos L., Sastre-Gómez A., García-Chico P., Palomino-Muñoz T.J. Impact of reference change value (RCV) based autoverification on turnaround time and physician satisfaction. Biochem Med (Zagreb) 2017, 27(2):342–9.*
23. *Ricós C., García-Victoria M., de la Fuente B. Quality indicators and specifications for the extra-analytical phases in clinical laboratory management. Clin Chem Lab Med 2004, 42(6):578–82.*
24. *Kenny D., Fraser C.G, Hyltoft P. Consensus agreement. Scand J Clin Lab Invest. 1999, 59(7):585–585.*
25. *Fraser C.G. Reference change values. Clin Chem Lab Med 2011, 50(5):807–12.*
26. *Iglesias Canadell N., Hyltoft Petersen P., Jensen E., Ricós C., Jørgensen P.E. Reference change values and power functions. Clin Chem Lab Med 2004, 42(4):415–22.*
27. *Biosca C., Ricós C., Lauzurica R., Galimany R., Hyltoft Petersen P. Reference change value concept combining two delta values to predict crises in renal posttransplantation. Clin Chem 2001, 47(12):2146–2148.*
28. *Walton R.M. Subject-based reference values: biological variation, individuality, and reference change values. Vet Clin Pathol 2012, 41(2):175–81.*
29. *Lund F., Petersen P.H., Fraser C.G., Sölétormos G. Calculation of limits for significant bidirectional changes in two or more serial results of a biomarker based on a computer simulation model. Ann Clin Biochem 2015, 52(Pt 4):434–40.*
30. *Fraser C.G. Inherent biological variation and reference values. Clin Chem Lab Med 2004, 42:758-764.*
31. *Flatland B., Baral R.M., Freeman K.P. Current and emerging concepts in biological and analytical variation applied in clinical practice. J Vet Intern Med 2020, 34(6):2691–700.*

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Review
Pregledni rad

FIZIOLOŠKE ULOGE, FARMAKOKINETIKA I FARMAKODINAMIKA NIACINA KOD KRAVA

PHYSIOLOGICAL ROLE, PHARMACOKINETICS AND PHARMACODYNAMICS OF NIACIN IN COWS

Kosta Petrović[a]*, Dragica Stojanović[b], Filip Štrbac[c], Talija Hristovska[d]

[a]Poljoprivredna škola sa domom učenika Futog, Srbija
[b]Departman za veterinarsku medicinu, Poljoprivredni fakultet Novi Sad, Univerzitet u Novom Sadu, Srbija
[c]Institut za multidisciplinarna istraživanja, Univerzitet u Beogradu, Srbija
[d]Veterinarski fakultet Bitolj, Univerzitet "Sveti Kliment Ohridski", Severna Makedonija
*Autor za kontakt: kostapetrovic84@gmail.com

D.S. https://orcid.org/0000-0003-4781-2623, F.Š. https://orcid.org/0000-0002-2666-7992, T.H. https://orcid.org/0000-0003-3892-4375

SAŽETAK

Vitamin B3 (Niacin, Vitamin PP) je spoj dve hemijske strukture, nikotinske kiseline, tj. niacina i nikotinamida. Niacin se ne svrstava direktno u vitamine i jer ih organizam sintetiše u normalnim metaboličkim uslovima i skoro potpuno zadovoljava telesne potrebe. Uzimajući u obzir da za njegovu proizvodnju kod krava mora imati dovoljno triptofana i mora postojati dovoljan unos hrane i uslovi u buragu, niacin je voma značajan vitamin koji se mora dodavati. Niacin je izuzetno važan za veliki broj metaboličkih i energetskih aktivnosti, a prekursor niacina je aminokiselina triptofan. Biohemijska funkcija nikotinske kiseline i nikotinamida se ostvaruje kroz funkciju koenzima, jer ovi vitameri ulaze u sastav koenzima NAD+ i NADP+, koji su sastojci oko 150 različitih enzima, otuda je razumljiva važnost niacin za energetski metabolizam. Važniji metabolički procesi za koje se vezuje uticaj niacina psredstvom koenzima su anabolički procesi poput, sinteze nukleinskih kiselina i lipida, gde je NADPH redukujući činilac. U kataboličkim procesima treba istaći učestvovanje NAD u većem broju oksidoredukcionih reakcija, u kojima je uloga NAD prenošenje elektrona iz najvišeg energetskog nivoa, po čemu su ove reakcije poznate. Niacin pokazuje antilipolitičko delovanje, utiče na metabolizam ugljenih hidrata i smanjuje insulinsku rezistenciju, a ima i antiinflamatorni efekat. Sve navedeno ukazuje da je niacin značajan kod krava u metaboličkom stresu, pa je u ovom radu detaljno opisana i njegova farmakokinetika i farmakodinamika.

Ključne reči: krave, niacin, metabolički stres, antilipolitički efekat, inflamacija.

ABSTRACT

Vitamin B3 (Niacin, Vitamin PP) is a combination of two chemical structures, nicotinic acid, i.e. niacin and nicotinamide. Niacin is not classified directly as a vitamin because the body synthesizes it under normal metabolic conditions and almost completely satisfies the body's needs. Considering that for its production in cows it must have enough tryptophan and there must be sufficient food intake and conditions in the rumen, niacin is a very important vitamin that must be supplemented. Niacin is extremely important for a large number of metabolic and energetic activities, and the precursor of niacin is the amino acid tryptophan. The biochemical function of nicotinic acid and nicotinamide is realized through the function of coenzymes, because these vitamers are included in the composition of coenzymes NAD+ and NADP+, which are components of about 150 different enzymes, hence the importance of niacin for energy metabolism. More important metabolic processes that are linked to the influence of niacin through coenzymes are anabolic processes such as the synthesis of nucleic acids and lipids, where NADPH is the reducing agent. In catabolic processes, it should be emphasized the participation of NAD in a greater number of oxidation-reduction reactions, in which the role of NAD is to transfer electrons from the highest energy level, which is what these reactions are known for. Niacin shows antilipolytic action, affects carbohydrate metabolism and reduces insulin resistance, and has an anti-inflammatory effect. All of the above indicates that niacin is important in cows under metabolic stress, so this paper describes its pharmacokinetics and pharmacodynamics in detail.

Key words: cow, niacin, metabolic stress, antilipolytic effects, inflammation.

FIZIOLOŠKE ULOGE NIACINA I NJEGOVA PRIMENA KOD PREŽIVARA

Kriterijumi koje neki nutritijent mora da ispunjava, da bi se mogao smatrati vitalnim su : jasan uticaj na metabolizam u vrlo maloj količini (dijetetski bitan), nasuprot čega stoji da ako nastane deficit u hrani i te male količine, kroz određeno vreme nastaje karakteristična bolest sa simptomima koji prate deficit određenog vitamina. Nasuprot, vraćanje u hranu deficitarnog vitamina treba da rezultira eliminacijom simptoma koji karakterišu bolest nastalu usled deficita tog vitamina. D vitamin i Niacin su na neki način prelazna forma u kategoriji nutritijenata jer ne ispunjavaju veoma stroge kriterijume koji standardizuju vitamine, ali se ipak svrstavaju u vitamine jer imaju karakteristične deficitarne bolesti (rahitis i pelagra). U vitamine se ne svrstavaju direktno i zbog činjenice da ih u normalnim metaboličkim uslovima organizam sam sintetiše i skoro potpuno zadovoljava telesne potrebe (1). Različita hemijska jedinjenja koja pokazuju istu biološku aktivnost, poznata su kao vitameri. Hemijska formula nikotinske kiseline je, C6H5NO2, naziv nosi po otrovnom amiloidu nikotinu i čini njegovo sastavni deo. Kada se govori o niacinu, misli se na dva njedova oblika, prvi je 3-piridinkarboksilna kiselina što je zapravo, nikotinska kiselina, a drugi je piridinkarboksiamid, nikotinamid, otuda i veći broj naziva za ovaj vitamin, te se često može čuti i naziv amid nikotinske kiseline, vitamin PP, pelagrin ili antipelagra vitamin. Nikotinska kiselina je blago kiselkastog ukusa, bez mirisa, nalazi se u obliku kristala, rastvara se u vodi praveći zasićen vodeni rastvor. Niacin je izuzetno važan za veliki broj metaboličkih i energetskih aktivnosti, a prekursor niacina je aminokiselina triptofan. Biohemijska funkcija nikotinske kiseline i nikotinamida se ostvaruje kroz funkciju koenzima, jer ovi vitameri ulaze u sastav koenzima NAD+ i NADP+, koji su sastojci oko 150 različitih enzima, otuda je razumljiva važnost niacin za energetski metabolizam. Važniji metabolički procesi za koje se vezuje uticaj niacina psredstvom koenzima su anabolički procesi poput, sinteze nukleinskih kiselina i lipida, gde je NADPH redukujući činilac. U kataboličkim procesima treba istaći učestvovanje NAD u većem broju oksidoredukcionih reakcija, u kojima je uloga NAD prenošenje elektrona iz najvišeg energetskog nivoa, po čemu su ove reakcije poznate. S obzirom da NAD elektron prenosi u oba smera, NAD se ponaša kao oksidaciono i redukciono sredstvo, jer može da prima i da otpušta elektron. Kada ima ulogu redukcionog sredstva NAD otpušta elektron i postaje NAD+, pri čemu redukuje target supstancu, kada ima ulogu oksidacionog sredstva tada prima elektron te postaje NADH, oksidujući supstancu sa kojom je stupio u reakciju. Niacin učestvuje u modifikaciji proteina, jer je izvor adenin dinukleotid fosfata, (ADP)-riboze. Dalje u kaskadnoj reakciji, ADP je precursor za dva sekundarna mesindžera, to su nikotinska kiselina adenindinukleotidfosfat i CADP-riboza, oba mesindžera stimulišu ulazak Ca+jona u ćelije, odakle je potuno jasno zašto se veći deo metabolizma nalazi pd direktnom, ili indirektnom kontrolom niacina. Utvrđeno je da aplikacija nikotinske kiseline u višestrukoj dozi, dovodi da zaustavljanja procesa razlaganja masti (2). U organizmu životinja, niacin se nalazi u dva oblika: nikotinska kiselina(u većem procentu), a drugi oblik je nikotinamid. Oba oblika su važni nutritijenti sa sličnim karakteristikama, oba oblika su takođe prekursori za sintezu NAD-a, ali su im biološki efekti različiti, naime samo nikotinamid može biti reaktivan (3). Poznato je da životinje same mogu sintetizovati niacin preko enzimske transformacije triptofana i hinolonske kiseline koji su prekursori niacina. Kod preživara mikroorganizmi u rumenu takođe sintetizuju niacin iz prekursora, sa razlikom što im je izbor prekursora mnogo veći, te tako mogu koristiti aspartate, kao i dihidroksiaceton fosfat (4). Uzimajući u obzir ovu čiunjenicu, treba obratiti pažnju na sastav hrane kojom se životinje hrane, jer nisu sva hraniva dovoljno bogata triptofanom, na prvom mestu kukuruz, a poznato je da kukuruz čini veći deo koncentrovanog obroka kod preživara. Treba napomenuti da niacin ima i prirodnog antagonistu, a to je antivitamin piridin-3-sulfonska kiselina. Antivitamini su takva jedinjenja, da se ugrađuju na mesto ugradnje agonista, odnosno

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vitamina, u ovom slučaju zauzimaju mesto vitaminima na mestu ugradnje u koenzime. Anti-vitamini inhibiraju rast mikroorganizama te i tako izazivaju avitaminoze. Piridin-3-sulfonska kiselina se nalazi u zrnu kukuruz, to je još jedan od brojnih ratloga da se preživarima ukoliko im se ishrana bazira na kukuruzu, u obrok dodaje niacin i triptofan. Ranija saznanja bazirana na istraživanjima u prošlosti, upućuju da se mlečnim kravama ne dodaju suplementi na bazi B-vitaminskih kompleksa, jer se smatralo da buražna mikroflora podmiruje sve potrebe za vitaminima B kompleksa (5). Danas se mlečnost u mlečnom govedarstvu toliko povećala, da mikroorganizmi iz predželudaca ne mogu obezbediti dovoljne količine B vitamina, pa se moraju dodavati ishrani u vidu suplemenata. Poznato je da se u peripartalnom periodu unos hrane značajno smanjuje, a potrebe za nutritijentima značajno povećavaju, što upućuje na još jedan razlog za dodavanje niacina ishrani. Niacin namenjen za oralnu ishranu kod krava nalazi se u dva oblika, jedan oblik je nezaštićen od razgradnje u buragu, dok drugi ima zaštićenu formulaciju, kojom se izbegava razgradnja u buragu (6). Nezaštićeni oblici niacin zbo razgradnje u rumenu, iziskuju veću dozu a i više koštaju, te nisu prvi lek izbora. Zaštićeni oblici su u formi kapsule, inkapsulirani su u lipidnu kapsulu koju sačinjavaju lipidi u više slojeva, time se postiže izbegavanje razgradnje u svim predželucima, oslobađanje niacina dešava se tek u tankom crevu gde mu je bioraspoloživost najveća.

ABSORPCIJA I RASPODELA NIACINA

Budući da je u ovom izlaganju primarno reč o mlečnim kravama, akcenat u vezi sa metabolizmom niacna, odnisi se na specifičnosti metabolizma niacina kod preživara (7-14). Naime specifičnosti o kojima je reč su vezane za anatomiju digestivnog trakta i predželudce kod preživara., jer farmakokinetika lekova kod oralne aplikacije obuhvata mogućnost dodatne razgradnje leka, što kod monogastričnih životinja nije moguće. Primena niacin kod krava je u vidu aplikacije oba njegova vitamera sa razlikom u farmakokinetici kod vitamera, zajedničko je da su obe forme prekursori za sintezu NAD-a. Mikrorganizmi kao što je već navedeno takođe sintetišu niacin, dnevna sinteza buražne mikroflore iznosi 2,2 g. Neka istraživanja ističu da ishrana i razlike u sastavu obroka, koa i suplementacija krava sa niacinom, određuju koliku količinu niacina će stvoriti mikroorganizmi, navodi se da ukoliko je dodatak niacina u ishranu veći, količina koju mikrorganizmi sintetišu se smanjuje. Nađeno je da se niacin kod preživara u znatnoj meri sintetiše i da se veći deo nikotinske kiseline i sva količina nikotinamida skladišti u samim bakterijama. Poznato je da se u buragu dešava apsorpcija niacina, ali uz ograničenja direktne apsorpcije, jer je njveći deo niacina vezan za buražne saprofite, a deo nikotinske kiseline koji je slobodan zbog niskog pH nalazi se u obliku jona. Generalno kada se razmatra ovaj vid apsorpcije, dolazi se do saznanja da su svi vidovi apsorpcije u buragu mali i ograničeni, i da je apsorpcija u tankom crevu, kao i svih ostalih nutritijenata osnovni put unosa. U istraživanjima u kojima je aplikovan niacin bez zaštite od razgradnje, došlo se do saznanja da je samo 17 %, od ukupne peroralne doze stiglo da tankog creva. Kod mlečnih krava razgradnja niacina u buragu iznosi 98 %, dok je kod tovnih goveda 93,8 %. Naslanjajući se na ove navode, neki istraživači su višestruko povećali dozu niacina do 36g na dan, dok je prethodna doza bila tri puta manja i iznosila je svega 12g po danu. Ovim istraživanjem se pokazalo da veća doza niacina zadovoljava sve potrebe mikroorganizama, te se i ostatak povećava i može dospeti u tanko crevo. Najpre količina niacina koja stigne do duodenuma prvenstveno zavisi od farmakokinetike i formulacije niacina (zaštićen i nezaštićen oblik). Nikotinska kiselina kada se nalazi u nezaštićenom obliku (ima jedan nesparen elektron viška), samo je 5% bioraspoloživa. Fiziološki put i mehanizam apsorpcije niacina ispitivan je na ljudima i pacovima, nađeno je da apsorpcija zavisi od pH i Na^+ jona-nosača. Kada se koncentracija aplikovanog niacin kod pacova poveća, glavni mehanizam apsorpcije je difuzija. U duodenumu pomoću enzima NAD-glukohidrolaze koji se nalazi u mukozi, konvertuje se nikotinska kiselina, ali ne direktno u nikotinamid, nego preko prelaznih jedinjenja. Nađeno je, da se kod mlečnih krava oblik niacina u formi nikotinamida sto puta veći nego što je u obliku nikotinske kiseline. Nikotinamid je glavna forma niacina u krvi, a dejstvo na organizam ispoljava preko svoja dva koenzima NAD i NADP. Za transport niacina u krvi veruje se da su odgovorni eritrociti, ali je njegovo zadržavanje u krvi vrlo kratko, jer su target tkiva u koja odlazi bubrezi, jetra i masno tkivo, sa primatom jetre, jer se u njoj odigravaju svi metabolički procesi, te je u jetri koncentracija niacina najveća.

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NIKOTINAMID-ADENIN-DINUKLEOTID KOENZIMI

Nikotinamid je reaktivni deo NAD-a i NADP-a poznatih koenzima, za koje se zna da ulaze u veliki broj enzima. Enzimi u čiji sastav ulaze NAD i NADP, učestvuju u brojnim oksdativnim i redukcionim reakcijama, odnosno učestvuju u metabolizmu ugljenih hidrata, proteina i masti. NAD i NADP su odgovorni za transfer vodonika u ćelijama, koji se oslobađa prilikom razlaganja šećera, masnih kiselina i aminokiselina. Najvažnije i najpoznatije anaboličke i kataboličke reakcije NAD-a i NADP-a su: metabolizam proteina (razgradnja i sinteza aminokiselina), oksidacija lanca ugljenika posredstvom ciklusa limunske kiseline, metabolizam ugljenih hidrata (aerobna i anaerobna oksidacija glukoze, Krebsov ciklus), metabolizam masti (razlaganje i sinteza glicerola, oksidacija i sinteza masnih kiselina, sinteza steroida). Prekursori za sintezu NAD i NADP su: oba vitamera niacina (nikotinska kiselina i nikotinamid), aminokiselina triptofan, hinolonske kiseline. Jetra u svojim anaboličkim procesima za sintezu NAD i NADP, kao prekursor najviše koristi triptofan, posredstvom hidrolize, te se tako niacin sačuva za metaboličke procese u ekstra hepatičnim tkivima. U normalnim fiziološkim procesima ekskrecija i eliminacija nikotinske kiseline i nikotinamida gotovo da ne postoji, jer se oba vitamera niacin reapsorbuju u bubrežnim tubulima iz glomerularnog filtrata. U slučaju povećanog unosa niacin i prezasićenja, može doći do ekskrecije posredstvom bubrega, a oblici koji se ovim putem eliminišu su: N1-metilnikotinamid i metalpiridonkarboksamid (glavni metaboliti niacina u urinu). Koenzim NAD ulazi u sastav brojnih enzima a najpoznatiji su : NAD-glukohidrolaza, NAD-pirofosfataza, ADP-riboziltransferaza, poli(ADP-riboza) i polimeraza.

SPECIFIČNOSTI UTICAJA NIACINA NA METABOLIZAM LIPIDA KOD KRAVA

Niacin u buragu gde najpre počinje delovanje nakon unosa putem hrane, ima izuzetno povoljan i blagotvoran efekat na mikroorganizme buraga, s tim da najviše deluje na protozoe. Naime protozoe unose niacin i koriste za svoje metaboličke procese, te je za njih je vrlo značajan i ima esencijalno svojstvo, jer ga protozoe ne mogu same sintetisati, pa se u optimalnom prisustvu niacina njihov broj značajno povećava (15). Kada je o amonijaku reč, nađena su oprečna mišljnja iz literature, prema jednim navodima koncentracija amonijaka se povećava u prisustvu niacina, dok se prema drugim smanjuje nakon aplikacije niacina (16). Ipak zaključak je jasan da sa povećanjem broja buražne mikroflore a naročito protozoa, raste i koncentracija amonijaka u predželucima, jer poznato je da amonijak nastaje metaboličkom aktivnosti mikroorganizama.

Neesterifikovane masne kiseline su glavna komponenta triglicerida, koji su izgrađeni od trougljeničnog alkohola glicerola, i tri masne kiseline pojedinačno vezane za svaki ugljenikov atom. Trigliceridi su glavni depo masti u organizmu, a njihovom hidrolizom koju izaziva hormon zavisna lipaza, oslobađaju se sastavne komponente, glicerol i masne kiseline, poznatije kao NEFA. U peripartalnom periodu koncentracija NEFA u krvi raste, a to je rezultat negativnog energetskog bilansa, posledično je koncentracija NEFA u jetri takođe povećana, i veća od one koja može da se oksidiše, te se višak ponovo u obliku triglicerida skladišti u jetri, ali i mišićima. Rezultat je pad apetitia i smanjena konverzija hrane. Ako se nikotinska kiselina primeni u višestrukoj dozi, otežava mobilisanje masti iz depoa (17,18,19). Antilipolitički efekat niacina je dokazan na adipoznom tkivu pacova, davne 1963 od kada je podatak poznat (3). Ispitivanja koja su vršena na živim životinjama, pokazuju da primena nikotinske kiseline u farmakološkim dozama, smanjuje koncentraciju slobodnih NEFA u krvi, jer zaustavlja lipolizu (20). Ovaj efekat nikotinske kiseline verovatno se dešava dejstvom na nikotinski receptor GPR109A. Nasuprot ovoj tvrdnji nikotinamid ima malu mogućnost vezivanja za GPR109A receptor, koji je verovatno zadužen samo za nikotinsku kiselinu (21). Kada nikotinska kiselina aktivira GPR109A receptor, inhibira se aktivnost adenilat ciklaze i smanjuje se koncentracija cAMP u ćelijama, te se posledično inaktiviše protein kinaza A, i smanji se fosforilacija hormone osetljive lipase, te se i lipoliza smanjuje (22). Mesta aktivacije GPR109A receptora kod goveda su : jetra, mišići, mozak, ali i adipozno tkivo koa i imunološke ćelije (20). Endogeni ligand za GPR109A kod ljudi je BHB, nikotinamid je slab agonist, dok su kod goveda za ovaj receptor endogeni ligandi brojni a to su : nikotinamid i nikotinska kiselina, kao i BHB svima je krajnji cilj smanjenje lipolize.Naime najefikasniji antilipolitički efekat ima nikotinska kiselina, kod nikotinamida ovakav efekat je dokazan samo in vitro uslovima na tkivu goveda, dok BHB u

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višestrukoj koncentraciji smanjuje fosforilaciju hormon zavisne lipaze i oslobađanje glicerola (23). Pokazano je da nikotinska kiselina kod goveda ima snažan antilipolitički efekat, što može biti od koristi za primenu u peripartalnom periodu i negativnom energetskom bilansu (17,18,19). Ovo se tumači kao mogućnost, jer u obzir treba uzeti sve metaboličke procese i potrebe nastupajuće laktacije, te tako odrediti optimalne doze nikotinske kiseline, koja se najbolje apsorbuje u tankom crevu (10). Aplikacija nikotinske kiseline, ostvaruje pozitivan efekat kod krava u vreme tranzicije, utvrđeno je da optimalna doza iznosi 12g /danu inkapsuliranog niacin (24). Posledica visoke koncentracije NEFA pored masne jetre je ketoza sa stvaranjem ketonskih tela. Studije koje su ovaj problem istraživale, su pokazale da primena niacina smanjuje koncentraciju BHB i NEFA u plazmi, a nivo glukoze povećava (25). Zbog podređenosti organizma razvoju ploda i prioriteta upotrebe glukoze u stanju graviditeta i razvoja vimena kod krava nastaje insulinska rezinstencija. Kod mlečnih krava insulinska rezistencija se povezuje i sa povećanom koncentracijom NEFA u krvi (17,18,19). Zna se da niacin smanjuje lipolizu i podiže glikemiju, time može povećati efikasnost insulina i smanjiti insulinsku rezistenciju. Nikotinska kiselina podiže nivo glukoze u krvi, iako je i dalje mehanizam dejstva nepoznat (26). Pretpostavlja se da utiče na povećanje stvaranja glukoze u jetri, ili smanjuje klirens glukoze u krvi, ili ispoljava oba efekta, što je najverovatnije. Koncentracija insulina ukoliko prati dinamiku kretanja glukoze u krvi, upućuje na smanjenje insulinske rezistencije (20). Primećeno je da primena niacina u ishrani, sadržaj nikotinamida u mleku raste, ali kada se primeni maksimalna doza niacina, sadržaj nikotinamida stagnira pa čak i opada. Koncentracija niacina u rumenu nema korelacijski odnos sa vrednostima niacina u mleku, niti se prinos mleka pod dejstvom niacina menja (6). što se objašnjava pozitivnim energetskim bilansom, te tako niacin ne utiče na mlečnost.

Pod terminom gojaznost podrazumeva se poremećaj preteranog nakupljanja masnog tkiva u organizmu. Patofiziološki proces vezan za gojaznost, dešava se na nivou same masne ćelije, adipocita, naime unutar adipocita se nakupljaju trigliceridi, što podrazumeva njihovo skladištenje i čuvanje. Proces dalje rezultira hipertrofijom adipocita, a posledica hipertrofije je disregulacija sekrecije adipokina, tojest smanjena proizvodnja anti-inflamatornih adipokina od kojih je najpoznatiji adiponektin (27). Gojaznost na drugoj strani ima za posledicu povećanu proizvodnju proinflamatornih hemiokina, i citokina koji uključuju MCP-1 i TNF-α (28). Gojaznost takođe prati još, izražena inflamacija sa M-1 (proinflamatorni makrofagi), u masnom tkivu, koje i samo proizvodi inflamatorne citokine kojima se još jače potencira upala masnog tkiva, te se tako začarani krug koji se tiče upale konstantno održava i produbljuje. Kod ljudi i glodara dokazano je da se kod gojaznih jedinki nalazi veći broj makrofaga u odnosu na one koji su normalno uhranjeni, ili su mršavi (29). Makrofagi masnog tkiva (ATM), učestvuju u inflamatornim procesima koji se dešavaju unutar samog masnog tkiva (29) a identifikovane su dve različite populacije ATM-a. Prva ATM populacija označena je kao M1(klasično aktivirani proinflamatorni makrofagi), oni se pojačano nakupljaju u masnom tkivu kao direktna posledica gojaznosti, dok se druga populacija označava sa M2(alternativno aktivirani inflamatorni makrofagi), a mogu se naći u masnom tkivu samo kod mršavih i normalno uhranjenih ljudi i glodara. Poznato je da gojaznost izaziva fenotipsku promenu populacije makrofaga u adipoznom tkivu, iz anti-inflamatornog M2 stanja u stanje inflamacije M1 (30). Klasifikacija ATM-a i razdvajanje u dve različite populacije makrofaga je preciznije objašnjenje fiziološke dinamike kod gojaznosti, pri čemu su M1 i M2 krajnje suprostavljene vrednosti. Zapravo ATM se preciznije definiše kao M1 koji predstavlja proinflamatorne makrofage, dok se M2 opisuje sa M2a, M2b, M2c, pri čemu su M2a citokini, M2b označava hemiokine a M2c su površinski receptori (Mantovani i sar., 2004). Svaka od navedenih frakcija makrofaga ima specifičan mehanizam aktivacije, tako je naprimer aktivator M1 interferon i lipopolisaharidi, M2a aktiviraju interleukini IL4 i IL13, M2b aktiviraju imuni kompleksi i IL-1b, M2c IL10 i glukokortikoidi (31). Mehanizmi koji su u osnovi smanjene proizvodnje adiponektina, uključuju promene u hormonskom statusu, oksidativni stress i upalu. Glavni medijatori koji uzrokuju upalu masnog tkiva i smanjenje serumske koncentracije adiponektina, su upalni citokini IL6 i TNF-α. Naime u istraživanjima koja su sprovedena na ljudima, došlo se do saznanja da niacin ispoljava anti-inflamatorni efekat u endotelnim ćelijama krvnih sudova, monocitima, epitelnim ćelijama pluća i bubrega. Međutim malo studija je rađeno na domaćim životinjama, koja istražuju anti-inflamatorne efekte niacina u masnom tkivu i drugim tkivima. Studije koje su sprovedene kod miševa uzeto je šest jedinki muškog pola i stavljeno na režim kontrolisane ishrane, nakon 6 nedelja i perioda u kom su ogledni miševi hranjeni hranom koja je bogata sa mastima, počelo se sa tretmanom u kom se miševima svakodnevno aplikovao niacin u dozi od 200 mg /kg, dok je kontrolna grupa primala vodu. Tretman je trajao četiri nedelje, a u petoj nedelji je doza niacina sa 200 mg/kg, podignuta na 360 mg/kg/danu. Studija je pokazala da niacin ispoljava anti-inflamatorni efekat u masnom tkivu, bez uticaja na sadržaj makrofaga u masnom tkivu kod mršavih i normalno uhranjenih miševa, dok je kod gojaznih miševa niacin povećao koncentraciju anti-inflamatornog adiponektina za 21%. Tako je pokazano da niacin ispoljava značajan anti-inflamatorni efekat i kroz smanjenu ekspresiju pro-inflamatornih citokina, povećanu ekspresiju anti-inflamatornih citokina, i smanjenje sadržaja M1 makrofaga u masnom tkivu. Slična studija sprovedena je kod ljudi a dobijeni rezultati su vrlo slični onim dobijenim na miševima, jer je i kod ljudi pokazano da niacin dramatično povećava serumske koncentracije adipokina, adiponektina kod gojaznih muškaraca sa metaboličkim sindromom (32). Adiponektin je jedan od najperspektivnijih biomarkera metaboličkog sindroma i poseduje snažne insulin-senzibilizirajuće, anti-aterosklerotične i anti-inflamatorne osobine. Studije su pokazale da niacin ima anti-inflamatorne efekte u brojnim tkivima, a potvrđeno je u tkivu bubrega, pluća, masnom tkivu, monocitima, endotelu krvnih sudova i još nekim. Studije koje su ovo dokazale na masnom tkivu, su sprovedene na kultivisanim adipocitima, i tada je pokazano da tretman niacinom smanjuje TNF-α i sekreciju proinflamatornih hemiokina MCP-1, fraktalkina i rantesa, dok na drugoj strain povećava mRNA adiponektina (33), su pokazali da niacin inhibira vaskularnu upalu, smanjenjem produkcije endotelnih reaktivnih kiseonika i inflamatornih citokina (34). Niacin sistemski smanjuje visokosenzibilni C-reaktivni protein i TNF-α, a oba su glavni sistemski markeri upale (35).

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LITERATURA

1. *Bender D.A. Niacin. In: Nutritional biochemistry of the vitamins. 2ed ed. Cambridge: Cambridge University Press, 2003.*
2. *Tunaru S., Kero J., Schaub A., Wufka C., Blaukat A., Pfeffer K., Offermanns S. PUMA-G and HM74 are receptors for nicotinic acid and mediate its anti-lipolytic effect. Nat. Med. 2003, 9, 352–355.*
3. *Carlson L.A. Studies on the effect of nicotinic acid on catecholamine stimulated lipolysis in adipose tissue in vitro. Acta Med. Scand. 1963, 173:719–722.*
4. *Henderson L.M. Niacin. Annu Rev Nutr. 1983, 3, 289-307.*
5. *National Research Council. Nutrient Requirements of Dairy Cattle, 2001.*
6. *Di Costanzo A., Spain J.N., Spiers D. Supplementation of nicotinic acid for lactating Holstein cows under heat stress conditions. J. Dairy Sci. 1997, 80, 1200–1206.*
7. *Campebell J.M., Murphy M.R., Christensen R.A., Overton R.R. Kinetics of niacin supplements in lactating dairy cows, J. Dairy. Sci. 1994,77, 566-575.*
8. *Di Palma J.R., Thayer W.S. Use of niacin as a drug. Annu. Rev. Nutr. 1991, 11, 169.*
9. *Erickson P.S., Murphy M., Clark J.H. Supplementation of dairy cow diets with calcium salts of long-chain fatty acids and nicotinic acid in early lactation. J. Dairy Sci. 1992, 75, 1078.*
10. *Morey S.D., Mamedova L.K., Anderson D.E., Armendariz C.K., Titgemeyer E.C., Bradford B.J. Effects of encapsulated niacin on metabolism and production of periparturient dairy cows J.Dairy Sci. 2011, 94, 5090-5104.*
11. *Nabokina S.M., Kashyap M.L., Said H.M. Mechanism and regulation of human intestinal niacin uptake. Am. J. Physiol. Cell Physol. 2005, 289, 97-103.*
12. *Santschi D.E., Barthiaume R., Matte J.J., Mustafa A.F., Girard C.L. Fate of supplementary B-vitamins in gastrointestinal tract of dairy cows. J.Dairy Sci. 2005, 88, 2043-2054.*
13. *Santschi D.E., Chiquette J., Berthiaume R., Martineau R., Matte J.J., Mustafa A.F., Giard C.L. Effects of the forage to concentrate ratio on B-vitamin concentrations in different ruminal fractiones of dairy cows. Can. J. Anim. Sci. 2005, 85, 385-399.*

ISBN 978-86-7520-611-8

14. *Zinn R.A., Owens F.N., Stuart R.L., Dunbar J.R-, Norman B.B. B-vitamin supplementation of diets for feedlot calves. J. Anim. Sci. 1987, 65(1), 267-77.*
15. *Niehoff I.D., Hüther L., Lebzien P., Flachowsk G. The effect of a Niacin supplementation to different diets on ruminal fermentation and flow of nutrients to the duodenum of dairy cows. Appl. Agric. Forestry. Res. 2013, 63(2):143-154.*
16. *16.Riddell D.O., Bartley E.E., Dayton A.D. Effect of nicotinic acid on rumen fermentationin vitroand in vivo.J. DairySci. 1980, 63(9):1429-1436.*
17. *Pires J. A.A., Pescara J.B., Grummer R.R. Reduction of plasma NEFA concentration by nicotinic acid enhances the response to insulin in feed-restricted Holstein cows. J. Dairy Sci. 2007, 90:4635–4642.*
18. *Pires J.A.A., Grummer R.R. The use of nicotinic acid to induce sustained low plasma nonesterified fatty acids in feed-restricted Holstein cows. J. Dairy Sci. 2007, 90:3725–3732.*
19. *Pires J. A. A., Souza A. H., Grummer R. R. Induction of hyperlipidemia by intravenous infusion of tallow emulsion causes insulin resistance in Holstein cows. J. Dairy Sci. 2007, 90:2735–2744.*
20. *Titgemeyer E., Spivey K., Mamedova L., Bradford B. Effects of pharmacological amounts of nicotinic acid on lipolysis and feed intake in cattle. Int. J. Dairy Sci. 2011, 6:134–141.*
21. *Gille A., Bodor E.T., Ahmed K., Offermanns S.:Nicotic acid: Pharmacological effects and mechanisms of action. Ann. Rev. Pharmacol. Toxicol. 2008, 48:79-106.*
22. *Offermanns S. The nicotinic acid receptor GPR109A (HM74A or PUMA-G) as a new therapeutic target. Trends. Pharmacol. Sci. 2006, 27 (7):384-390.*
23. *Kenéz A., Locher L. , Rehage J., Dänicke S., Huber K. Agonists of the G protein-coupled receptor 109A-mediated pathway promote antilipolysis by reducing serine residue 563 phosphorylation of hormone-sensitive lipase in bovine adipose tissue explants. J. Dairy Sci. 2014, 97:3626–3634.*
24. *Yuan K., Shaver R.D., Bertics S.J., Espineira M., Grummer R.R. Effect of rumen-protected niacin on lipid metabolism, oxidative stress, and performance of transition dairy cows. J.Dairy Sci. 2012, 95:2673-2679.*
25. *Dufva G.S., Bartley E.E., Dayton A.D., Riddell D.O. Effect of niacin supplementation on milkproduction and ketosis of dairy cattle. J. Dairy Sci. 1983, 66, 2329–2336.*
26. *Stefan N., Stumvoll M., Bogardus C., Tataranni P.A. Elevated plasma nonesterified fatty acids are associated with deterioration of acute insulin response in IGT but not NGT. Am. J. Physiol. Endocrinol Metab. 2003, 284(6):1156-1161.*
27. *Arita Y., Kihara S., Ouchi N., Takahashi M., Maeda K., Miyagawa J., Hotta K., Shimomura I., Nakamura T., Miyaoka K., Kuriyama H., Nishida M., Yamashita S., Okubo K., Matsubara K., Muraguchi M., Ohmoto Y., Funahashi T., Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999, 257: 79-83.*
28. *Hotamisligil G.S., Arner P., Caro J.F., Atkinson R.L., Spiegelman B.M. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 1995, 95: 2409-2415.*
29. *Weisberg S.P., McCann D., Desai M., Rosenbaum M., Leibel R.L., Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003, 112: 1796-1808.*
30. *Lumeng C.N., Bodzin J.L., Saltiel A.R. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 2007, 117: 175-184.*
31. *Martinez F.O., Sica A., Mantovani A., Locati M. Macrophage activation and polarization. Front Biosci 2008, 13: 453-461.*
32. *Plaisance E.P., Grandjean P.W., Brunson B.L., Judd R.L. Increased total and high-molecular weight adiponectin after extended-release niacin. Metabolism 2008, 57: 404-409.*
33. *Digby J.E., McNeill E., Dyar O.J., Lam V., Greaves D.R., Choudhury R.P. Anti-inflammatory effects of nicotinic acid in adipocytes demonstrated by suppression of fractalkine, RANTES, and MCP-1 and upregulation of adiponectin. Atherosclerosis 2010, 209: 89-95.*
34. *Ganji S.H., Qin S., Zhang L., Kamanna V.S., Kashyap M.L. Niacin inhibits vascular oxidative stress, redox-sensitive genes, and monocyte adhesion to human aortic endothelial cells. Atherosclerosis 2009, 202: 68-75.*
35. *Kwon W.Y., Suh G.J., Kim K.S., Kwak Y.H. Niacin attenuates lung inflammation and improves survival during sepsis by downregulating the nuclear factor-kappaB pathway. Crit Care Med 2011, 39: 328-334.*

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Review
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INTRACELULARNI I EKSTRACELULARNI HSP70 KOD KRAVA – SLIČNOSTI I RAZLIKE U FIZIOLOŠKIM I PATOFIZIOLOŠKIM USLOVIMA

INTRACELLULAR AND EXTRACELLULAR HSP70 IN COWS - SIMILARITIES AND DIFFERENCES IN PHYSIOLOGICAL AND PATHOPHYSIOLOGY CONDITIONS

Miloš Ži. Petrović[a*], Radojica Djoković[a], Vladimir Kurćubić[a], Snežana Bogosavljević Bošković[a], Simeon Rakonjac[a], Milun D. Petrović[a]

[a]Agronomski fakultet Čačak, Univertzitet u Kragujevcu, Srbija
*Corresponding author: petrovic.milos87@yahoo.com

M.Ži.P. https://orcid.org/0000-0002-4707-6796, R.Dj. https://orcid.org/0000-0003-0900-3227, V.K. https://orcid.org/0000-0003-2016-7345, S.B.B. https://orcid.org/0000-0002-8200-1415, S.R. https://orcid.org/0000-0002-1232-1755, M.D.P. https://orcid.org/0000-0001-8187-0785

SAŽETAK

Proteini toplotnog šoka (*Hsp*) nazivaju se još i čaperoni / šaperoni predstavljaju proteine koji su neizostavni za pravilno formiranje polipeptidnog lanca; i imaju ulogu u njegovoj translokaciji u okviru ćelije. Hsp70 u ćelijama pomaže u ponovnom uspostavljanju nativne konformacije proteina koji su se denaturisali pod dejstvom različitih stresogena, tako što sprečava njihovu agregaciju, što za rezultat ima čuvanje ćelije od apoptoze i imaju antiinflamatorni efekat. Ovi proteini su klasifikovani na osnovu molekularne mase, a najznačajniji je heat shock protein 70 (Hsp70) molekularne mase oko 70 kDa, koji je označen kao "a master player in protein homeostasis". Koncentracija Hsp značajno raste prilikom izlaganja nekom stresoru koji potiče iz same ćelije ili iz spoljašnje sredine. Mnogi čaperoni su indukovani pod dejstvom visokih ambijentalnih temperatura, kada se razvija univerzalni odgovor toplotnog šoka (HSR), pa je zbog toga definisan naziv heat shock proteins. Intracelularni Hsp70 (iHsp70) pokazuje svoje protektivno i antiinflamatorno dejstvo. Indukovani iHsp70 štiti ćeliju od apoptoze tako što redukuje ili blokira aktiviranje kaspaza, vezuje se za apoptozu-indukujući faktor (AIF) i inhibira AIF-indukovanu kondenzaciju hromatina ili onemogućuje oštećenje mitohondrija i fragmentaciju jedra. On blokira morfološke promene kod ćelije koje su izazvane apoptozom indukovanom pomoću tumor necrosis faktora, a nađeno je da pomaže u reparaciji ćelije prilikom oštećenja koje je izazvano inflamacijom. Antiinflamatorni efekat iHsp70 ogleda se u tome što inhibira odgovor na lipopolisaharide i blokira produkciju inflamatornih medijatora kao što je faktor nekroze tumora alfa (TNF-α), a opisani su i drugi mehanizmi. Ekspresija gena za produkciju Hsp70 je dobro izučena kod preživara ili njihovih ćelijskih kultura koje su izlagani visokim ambijentalnim temperaturama, a višestruki porast iHsp70 u ćelijama daje bolju adaptaciju na toplotni stres. Izučavanje eHsp70 je postalo aktuelno zbog dostupnih dijagnostičkih kitova za određivanje njegove koncentracije, a najnoviji rezultati pokazuju da je on veoma koristan prediktor mortaliteta kod pacijenata u septičnom šoku. Hsp70 prelazi u ekstracelularni prostor na nekoliko načina: izlaskom iz nekrotičnih ćelija, pod delovanjem različitih stresnih faktora i inflamacije kod neoštećenih ćelija, može biti produkovan u jetri kao protein akutne faze, a opisan je i transport egzozomima i direktan kontakt sa lipidnom membranom ćelija. Proinflamatorni efekat eHsp70 ostvaruje tako što indukuje imunološke ćelije, što dalje indukuje sekreciju inflamatornih citokina (TNF-α, IL-1β, IL-6), inducibilnu azot oksid sintazu (iNOS) ekspresiju i nuklearnu translokaciju nuklearnog faktora-κB (NF-κB). Prema teoriji bilansa čaperona, što je vrednost eHsp70 viša u odnosu na iHsp70, to su njegova proinflamatorna delovanja izraženija. Ova hipoteza je potvrđena i kod mlečnih krava u peripartalnom periodu.

Ključne reči: krave, protein toplotnog šoka, inflamacija, metabolizam.

ABSTRACT

Heat shock proteins (Hsp), also called chaperones, are proteins that are indispensable for the proper formation of the polypeptide chain; and have a role in its translocation within the cell. Hsp70 in cells helps to re-establish the native conformation of proteins that have denatured under the influence of various stressogens, by preventing their aggregation, which results in protecting the cell from apoptosis and having an anti-inflammatory effect. These proteins are classified on the basis of molecular mass, and the most significant is heat shock protein 70 (Hsp70) with a molecular mass of about 70 kDa, which is designated as "a master player in protein homeostasis". The concentration of Hsp increases significantly when exposed to a stressor originating from the cell itself or from the external environment. Many chaperones are induced under the influence of high ambient temperatures, when the universal heat shock response (HSR) develops, which is why the name heat shock proteins was defined. Intracellular Hsp70 (iHsp70) shows its protective and anti-inflammatory effects. Induced iHsp70 protects the cell from apoptosis by reducing or blocking the activation of caspases, binding to apoptosis-inducing factor (AIF) and inhibiting AIF-induced chromatin condensation or preventing mitochondrial damage and nuclear fragmentation. It blocks cell morphological changes caused by tumor necrosis factor-induced apoptosis, and has been found to aid in cell repair of damage caused by inflammation. The anti-inflammatory effect of iHsp70 is reflected in the fact that it inhibits the response to lipopolysaccharides and blocks the production of inflammatory mediators such as tumor necrosis factor alpha (TNF-α), and other mechanisms have been described. The expression of the gene for the production of Hsp70 has been well studied in ruminants or their cell cultures exposed to high ambient temperatures, and the multiple increase of iHsp70 in the cells results in a better adaptation to heat stress. The study of eHsp70 has become relevant due to the availability of diagnostic kits for determining its concentration, and the latest results show that it is a very useful predictor of mortality in patients with septic shock. Hsp70 moves to the extracellular space in several ways: after leaving necrotic cells, under the action of various stress factors and inflammation in undamaged cells, it can be produced in the liver as an acute phase protein, and transport by exosomes and direct contact with the lipid membrane of cells have also been described. The pro-inflammatory effect of eHsp70 is realized by inducing immune cells, which further induces the secretion of inflammatory cytokines (TNF-α, IL-1β, IL-6), inducible nitric oxide synthase (iNOS) expression and nuclear translocation of nuclear factor-κB (NF-κB). According to the chaperone balance theory, the higher the value of eHsp70 compared to iHsp70, the more pronounced its pro-inflammatory effects. This hypothesis was also confirmed in dairy cows in the periparturient period.

Key words: cow, heat shock proteins, inflammation, metabolism.

DEFINICIJA PROTEINA TOPLOTNOG ŠOKA I NJIHOVA ULOGA

Proteini toplotnog šoka (eng., *Heat shock proteins, Hsp*) nazivaju se još i čaperoni / šaperoni predstavljaju proteine koji su neizostavni za pravilno formiranje polipeptidnog lanca; i imaju ulogu u njegovoj translokaciji u okviru ćelije. Hsp70 u ćelijama pomaže u ponovnom uspostavljanju nativne konformacije proteina koji su se denaturisali pod dejstvom različitih stresogena, tako što sprečava njihovu agregaciju, što za rezultat ima čuvanje ćelije od apoptoze i imaju antiinflamatorni efekat. Pored navedenog, novija istraživanja koji se tiču ekstraćelijskog, cirkulišućeg Hsp70 govore u prilog činjenici da ovaj šaperon ima proinflamatornu ulogu, slično citokinima, ali i da poboljšava nadzor protiv tumorskih ćelija.

Proteini toplotnog šoka (Hsp) su molekularni čaperoni koji imaju ključnu ulogu u održavanju homeostaze proteina u ćeliji (protostaza) (1). Oni sprečavaju pogrešno savijanje i agregaciju proteina, što se postiže njihovim delovanjem na intermedijere savijanja (2-4). Ovi proteini su klasifikovani na osnovu molekularne mase, a najznačajniji je heat shock protein 70 (Hsp70) molekularne mase oko 70 kDa, koji je označen kao "a master player in protein homeostasis" (5). Koncentracija Hsp značajno raste prilikom izlaganja nekom stresoru koji potiče iz same ćelije ili iz spoljašnje sredine. Mnogi čaperoni su indukovani pod dejstvom visokih ambijentalnih temperatura, kada se razvija univerzalni odgovor toplotnog šoka

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(HSR), pa je zbog toga definisan naziv heat shock proteins (6,7). Intracelularni Hsp70 (iHsp70) pokazuje svoje protektivno i antiinflamatorno dejstvo. Indukovani iHsp70 štiti ćeliju od apoptoze tako što redukuje ili blokira aktiviranje kaspaza, vezuje se za apoptozu-indukujući faktor (AIF) i inhibira AIF-indukovanu kondenzaciju hromatina ili onemogućuje oštećenje mitohondrija i fragmentaciju jedra (8,9). On blokira morfološke promene kod ćelije koje su izazvane apoptozom indukovanom pomoću tumor necrosis faktora, a nađeno je da pomaže u reparaciji ćelije prilikom oštećenja koje je izazvano inflamacijom (10,11). Antiinflamatorni efekat iHsp70 ogleda se u tome što inhibira odgovor na lipopolisaharide i blokira produkciju inflamatornih medijatora kao što je faktor nekroze tumora alfa (TNF-α), a opisani su i drugi mehanizmi (12). Ekspresija gena za produkciju Hsp70 je dobro izučena kod preživara ili njihovih ćelijskih kultura koje su izlagani visokim ambijentalnim temperaturama, a višestruki porast iHsp70 u ćelijama daje bolju adaptaciju na toplotni stres (13-15).

INTRACELULARNA I EKSTRACELULARNA ULOGA HSP70

Hsp70 se deketuje u ćelijama, ali i u krvnom serumu, a u zavisnosti od lokalizacije on ima sposobnost da ispolji potpuno drugačije fiziološke efekte (16). Hsp70 u cirkulaciju dolazi putem dva mehanizma. Prvi mehanizam podrazumeva pasivan ulazak Hsp70 u krvotok koji je poreklom od nekrotičnih ili stresom opterećenih ćelija, dok drugi mehanizam uključuje aktivno lučenje Hsp u krvotok od strane različitih ćelija (17-19). Nema jasnih saznanja o tome koji deo Hsp u krvotok dolazi aktivnim, a koji deo pasivnim putem. Kontradiktorna uloga i ekspresija Hsp u različitim patofiziološkim procesima i stanjima dodatno otežava ovu interpretaciju. Naime, njegova koncentracija raste u različitim bolestima, a sa druge strane povećanje koncentracije znači i bolje preživljavanje, dok u određenim okolnostima smanjena produkcija Hsp dovodi do različitih metaboličkih poremćaja kod ljudi (20). Hsp70 stimuliše imuni odgovor, ali i vrši njegovu inhibiciju da previše jaka imunološka stimulacija ne bi dovela do oštećenja tkiva domaćina. Njegova uloga u patologiji ali i protekciji ćelija, te razlika u koncentracijama i ekspresiji u različitim bolestima i dalje zbunjuje kada pokušamo da formiramo jedinstvenu zakonitost o delovanju pa i poreklu Hsp70 u sistemskoj cirkulaciji.

Intracelularni HSP (iHsp70) ima snažan antiinflamatorni efekat, dok ekstracelularni HSP (eHsp70) ima suprotnu ulogu, indukujući aktivaciju nekoliko proinflamatornih puteva. Ekstracelularni eHsp70 dolazi u krvotok iz živih ćelija koje su izložene stresu najverovatnije putem putem vezikularne sekrecije, egzozoma ili lizozoma i preko intaktne lipidne membrane koji su nezavisni od transporta proteina preko sistema endoplazmatski retikulum-Goldžijev aparat, dok pasivnim putem dolazi iz oštećenih i nekrotičnih ćelija (21). Više inflamatornih puteva može biti pokrenuto kao posledica izloženosti ekstracelularnom eHsp70, najverovatnije putem njegovog vezivanja za Toll receptore na membranama ćelija (22,23). Sa druge strane, intracelularni iHsp70 vrši blokiranje aktiviranja nuklearnog faktora κB (NF-κB), čime postiže svoj antiinflamatorni efekat (24). NF-κB je opšti transkripcioni faktor koji je neophodan za pokretanje inflamatornih odgovora na različite signale, a otkriven je u B-limfocitima (25-27). Kod inflamatornog odgovora jetre, kao značajnog organa za proizvodnju proteina akutne faze i citokina od velikog značaja je povezivanje iHSP70 sa NF-κB/IKB kompleksom u citosolu hepatocita čime se sprečava transkripcija TNFα i inducibilnih gena azot-oksid sintaze, čime se postiže antiinflamatorni efekat šaperona (28). Preživljavanje ćelija je omogućeno posle inhibicije c-Jun N-terminal kinase-(JNK-) transdukcije signala koju vrši intracelularni Hsp70 posle njegovog povećanog stvaranja tokom stresnog odgovora (29). Zaštita ćelija u osnovi ima inhibiciju apoptoze koja se postiže na nekoliko načina. iHSP70 sprečava aktiviranje kaspaza, povećava ekspresiju Bcl-2 i inhibira otpuštanje citohroma C, a kod infarkta mozga povećana ekspresija ovog proteina toplotnog šoka smanjeuje veličinu infarkta i apoptozu (30-33). Hsp70 smanjuje oksidativni stres (34), a ciklopenenonski prostaglandini (cp-PGs), koji pod određenim okolnostima mogu izazvati ekspresiju Hsp70 i na taj način postaju moćni antiinflamatorni medijatori (35-37). Intraćelijski Hsp70 pokazuje i interferenciju sa proinflamatornim citokinima, što se postiže na nivou gena gde region gena koji promiviše TNF-α sadrži HSF1 vezujuće mesto koje potiskuje TNFα transkripciju, što znači da će aktivacijom HSF1 doći do smanjene ekspresije TNFα, što može biti postupak antiinflamatornog delovanja (38,39). Međutim, ovaj proces je dvosmeran, pa TNFα može

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zaustaviti aktivaciju HSF1 (40-42). Zbog ovakvih relacija na genskom nivou indukcija Hsp72 (HSPA1A) smanjuje ekspresiju gena za citokine kao što su TNFα, IL-1, IL-12, IL-10 i IL-18 (43). Za razliku od intracelularnih iHsp70, dokazano je da eHSP70 ima velikog značaja u inflamatornim reakcijama, što ostvaruje putem transdukcije MyD88/IRAK/NF-κB signala posle vezivanja sa Tool-like receptorom 2 (TLR2) i TLR4, putem CD14-zavisnih reakcija (44,45), čime se promoviše urođena imunološka aktivacija (46). eHSP70 ima parakrinu ulogu u krvotoku (47). eHSP70 indukuje signale koji daju tipičan proinflamatorni odgovor uz povećano stvaranje NO i proinflamatornih citokina TNF-α i inteleukin 1 beta (IL-1β) (48). eHSP70 je u pozitivnoj korelaciji sa klinički značajnim pokazateljima inflamacije kao što su CRP, fibrinogen i broj monocita (49).

Hsp70 ima dvojnu ulogu u organizmu u zavisnosti od toga da li je lociran intra- ili ekstracelularno, tako da iHsp70 ima zaštitnu ulogu, a eHsp70 ima proinflamatornu ulogu. Izučavanje eHsp70 je postalo aktuelno zbog dostupnih dijagnostičkih kitova za određivanje njegove koncentracije, a najnoviji rezultati pokazuju da je on veoma koristan prediktor mortaliteta kod pacijenata u septičnom šoku (50). Hsp70 prelazi u ekstracelularni prostor na nekoliko načina: izlaskom iz nekrotičnih ćelija, pod delovanjem različitih stresnih faktora i inflamacije kod neoštećenih ćelija, može biti produkovan u jetri kao protein akutne faze, a opisan je i transport egzozomima i direktan kontakt sa lipidnom membranom ćelija (51,52). Proinflamatorni efekat eHsp70 ostvaruje tako što indukuje imunološke ćelije, što dalje indukuje sekreciju inflamatornih citokina (TNF-α, IL-1β, IL-6), inducibilnu azot oksid sintazu (iNOS) ekspresiju i nuklearnu translokaciju nuklearnog faktora-κB (NF-κB) (53). Prema teoriji bilansa čaperona, što je vrednost eHsp70 viša u odnosu na iHsp70, to su njegova proinflamatorna delovanja izraženija (54).

HSP70 I PERIPARTALNI PERIOD KOD KRAVA

Prvi radovi u određivanju Hsp70 u krvi kod mlečnih krava - Kada se radi o mlečnim kravama u našim uslovima je ispitivana koncentracija Hsp70 kod krava u peripartalnom periodu (55). Koncentracija Hsp70 iznosila je 3,2±0,93 ng/mL u prvoj nedelji posle tenjenja, odnosno 3,7±0,88 u drugoj, da bi u četvrtoj i osmoj nedelji iznosila oko 5,5 ng/mL. Vreme posle teljenja pokazuje značajan statistički uticaj na vrednost ovog čaperona u krvi ($F=15,8$; $p<0,01$). Nađeno je da je koncentracija Hsp70 statistički značajno niža u prvoj i drugoj nedelji posle teljenja u odnosu na četvrtu i osmu. Ove vrednosti kao i njihov trend slažu se sa ranijim rezultatima (56,57). Postoje brojni faktori koji utiču na koncentraciju Hsp70 u serumu krava, kao što su starost i stadijum laktacije. Koncentracija Hsp72 je viša u prvih 60 dana latacije u odnosu na kasniju laktaciju. Međutim, koncentracija Hsp72 je značajno niža kod krava pred partus i u prvim nedeljama posle partusa, da bi potom rasla. Ovo ukazuje na postojanje određenih specifičnosti u regulaciji ekstracelularnog Hsp72 kod krava u peripartalnom periodu. Kod mlečnih krava postoji pozitivna korelacija između vrednosti ekstracelularnog i intracelularnog Hsp72. U malom broju istraživanja ispitana je veza peripartalnog metaboličkog stresa sa vrednostima čaperona. Koncentracija NEFA (neesterifikovanih masnih kiselina) u peripartalnom periodu pokazuju pozitivnu korelaciju sa koncentracijom Hsp72. Cincović i Belić (55) su pokazali da je koncentracija Hsp70 bila značajno viša u nedeljama posle teljenja u odnosu na nedelju pre teljenja. Viša koncentracija NEFA i BHB (beta hidroksibutirata) nađena je u prvoj i drugoj nedelji posle teljenja u odnosu na ostale periode. Koncentracija Hsp70 pozitivno korelira sa vrednostima NEFA i BHB. Parcijalna korelacija pokazuje da su veze jače u prvoj i drugoj nedelji posle teljenja, što je period kada su lipidna mobilizacija i ketogeneza najizraženije. Koncentracija Hsp70 u prve dve nedelje posle teljenja je zavisna od nivoa lipidne mobilizacije i ketogeneze. Metabolički stres koji se odlikuje lipidnom mobilizacijom i ketogenezom povećava koncentraciju Hsp70 u krvi tokom rane laktacije.

Promena koncentracije Hsp70 u funkciji graviditeta i porođaja je opisana u funkciji brojnih fizioloških i patoloških faktora koji postoje u periodu oko porođaja (58). Koncentracija Hsp70 je niža tokom trudnoće, u odnosu na žene koje nisu trudne (59). Sam partus indukuje stvaranje Hsp, jer je kod ovaca utvrđeno da postoji povećana ekspresija Hsp72 mRNA u mišićnom sloju materice tokom jagnjenja (60). Kod žena u amnionskoj tečnosti takođe raste koncentracija ovog šaperona kod žena koje su imale fiziološki porođaj (61). Kada se radi o mlečnim kravama, Kristensen i sar. (62) su pokazali da stadijum laktacije utiče na koncentraciju HSP72 u krnoj plazmi krava. Koncentracija Hsp72 u plazmi je bila veća u ranoj laktaciji u poređenju sa ostalim delovima laktacije, mada ove razlike nisu bile statistički signifikantne. Posmatrajući prve postpartalne nedelje nalazimo nižu koncentraciju eHSP70 u prvih dve nedelje nakon teljenja u odnosu na 4. i 8. nedelju (63). Još uvek nisu u potpunosti razjašnjeni mehanizmi koji dovode do različitog uticaja perioda laktacije na stvaranje proteina toplotnog šoka. Jedan od rezultata ukazuje da smanjen unos hrane u odnosu na potrebe, što je karakteristika peripartalnog perioda kod krava. U tom periodu se dešavaju promene u metabolizmu ugljenih hidrata i masti koje mogu uticati na stvaranje Hsp u različitim tkivima. Nađeno je da prolongirano unošenje manje energije nego što je potrebno dovodi do povećane produkcije Hsp90, međutim nisu utvrđene promene u mesendžernoj RNK u leukocitima, dok je ekspresija Hsp70 u somatskim ćelijama bila smanjena. Dostupnost ugljenih hidrata tokom izlaganja stresu u vidu fizičkog napora smanjuje Hsp70 u cirkulaciji, što ukazuje da dostupnost glukoze kao glavnog energenta smanjuje ekstracelulatne proteine toplotnog šoka, pa samim tim i njihovo potencijalni proinflamatorni efekat (64). Sa druge strane, povećano stvaranje Hsp72 tokom toplotnog stresa dovodi do povećanog korišćenja glukoze u energetske svrhe i smanjuje akumulaciju lipida u hepatocitima (65). Koncentracije Hsp72 u leukocitima i plazmi brzo rastu posle teljenja i koreliraju sa koncentracijama NEFA, glukoze i TNFα (66). Povezanost sa osnovnim metaboličkim procesima i inflamatornim odgovorom dovodi do razmatranja značaja Hsp u procesu nastanka insulinske rezisitencije, koja se nalazi u osnovi metaboličkih promena kod krava u peripartalnom periodu. Kod ljudi obolelih od dijabetesa tipa 2 postoji smanjena ekspresija mRNA za intracelularni Hsp72 u skeletnim mišićima, dok terapije i procedure koje podižu nivo ovog šaperona prevenira nastanak hiperglikemije, hiperinsulinemije, intolerancije na glukozu i insulinske rezistencije kod gojaznosti (67-69). Na modelu gojaznosti je ispitan i uticaj hronične inflamacije koja prati povećanu zastupljenost masnog tkiva. Naime, pokazano je da inflamacija uz stalnu aktivaciju NLRP3 vrši supresiju HSF-1/iHSP70 ose, čime se smanjuje ekspresija HSF-1 sa posledičnim povećanim ćelijskim starenjem i bržim propadanjem. Na osnovu navedenog zaključujemo da intracelularni ptotein toplotnog šoka Hsp70 ima antiinflamatorno i proinsulinsko delovanje. Ekstracelularni Hsp70 pokazuje suprotne fiziološke efekte pa je u tesnoj vezi sa razvojem inflamacije i diabetes mellitus-a tipa 2. Skeletni mišići i adipozno tkivo kao predstavnici insulin zavisnog tkiva imaju redukovane vrednosti iHsp70 tokom dijabetesa, uz povišene vrednosti eHsp70 (70-73). Kod krava u ranoj laktaciji postoji nedostatak insulina koji je posledica započinjanja laktacije i negativnog energetskog bilansa praćenog sniženom koncentracijom glukoze, što dalje dovodi do povećane lipolize, inflamacije i insulinske rezistencije. Kod krava sa povišenim vrednostima NEFA i sniženim vrednostima insulina nađena je viša koncentracija eHsp70 u nedenjama posle teljenja (74). Sve navedeno govori u prilog našoj hipotezi da je Hsp70 značajno uključen u sve metaboličke tokove i adaptacije kod krava u ranoj laktaciji.

Peripartalni period kod mlečnih krava predstavlja model za izučavanje metaboličkog stresa. Metabolički stress kod krava u ranoj laktaciji nastaje kao posledica teljenja i započinjanja laktacije, kada dolazi do promena u metabolizmu kako bi se podržala nadolazeća laktacija. Metabolički stres se odlikuje negativnim energetskim bilansom, povećanom proizvodnjom mleka, usmeravanjem glukoze ka mlečnoj žlezdi, dok se zadovoljavanje energetskih potreba drugih tkiva omogućuje kroz lipolizu i ketogenezu, koji utiču na celokupnu metaboličku adaptaciju krava (75,76). Povećan dotok masnih kiselina u jetru, uz pad lipoproteinskog transporta u organizmu dovodi do razvoja masne jetre kod krava i razvoja ketoze (75). Jedini lipogeni hormon u organizmu krava je insulin, čije vrednosti opadaju, a njegovo dejstvo biva inhibirano pod uticajem izražene lipolize i ketogeneze, pa se javlja insulinska rezistencija, koja dodatno stimuliše lipolizu (76). Nastanak lipolize, masne jetre i insulinkse rezistencije kod mlečnih krava je usko povezano sa nastankom inflamacije u ranoj laktaciji. Naime, povećana lipoliza kod krava može dovesti do odavanja veće količine proinflamatornih citokina iz masnog tkiva koji se nazivaju adipokini, a najvažniji je TNF-α, što je u vezi sa razvojem insulinske rezistencije i povećanjem aktivnosti lipaza (77). Nađena je povećana aktivnost TNF-α u serumu kod krava sa umerenim do teškim sindromom masne jetre (78). U više eksperimentalnih studija pokazan je direktni uticaj neesterifikovanih masnih kiselina (NEFA) na inflamatorne procese tako što utiče na aktivaciju toll-like receptora (TLR), posebno TLR4. TLR4

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aktivacija može da dovede do inflamatornog odgovora uz lučenje proinflamatornih citokina (79,80). Period laktacije i proizvodnja mleka kod krava utiču na vrednosti eHsp70. Tako je nađeno da posle partusa u ranoj laktaciji i sa porastom proizvodnje mleka raste koncentracija eHsp70 u krvnom serumu i salivi kod krava (81-83). Dinamičke promene u vrednosti eHsp70 i TNF-α su gotovo identične kod krava u prvim nedeljama posle teljenja kada postoji metabolički stres, tako da vrednosti oba parametra rastu. Takođe, Hsp70 utiče na mnoge metaboličke tokove koji se tiču energetskog metabolizma, metabolizma lipida u jetri i insulinske rezistencije, a svoje dejstvo ispoljava preko TLR4 (84,85).

Hsp70 ima velik iznačaj u procesu metabolizma lipida i njegova pozicija je dobro izučena u procesima autofagije i lipolize (86,87). Autofagija je ćelijski proces u kome se proteini i organele razgrađuju kroz lizozome kako bi se održala adekvatna ćelijska homeostaza. Postoje tri načina kako se ostvaruje autofagija: makroautofagija, mikroautofagija i autofagija posredovana šaperonom. Makroautofagija i šaperonima posredovana autofagija zajedno sa sistemima proteaze uključeni su u proces recikliranja ćelijskih proteina. Na ovaj način se regulišu i neutrališu i oni proteini koji mogu da ugroze ćeliju, jer su rezultat genskih aberacija i mutacija. Makroautofagija, ili autofagija u klasičnom smislu, nastaje posle formiranja autofagozoma, a to je dvomembranska vezikularna struktura koja uključuje ono što je potrebno da se razgradi. Posle formiranja autofagozoma, on se spaja sa lizozomom, gde se oslobađa proteolitički materijal kako bi se formirala autolizozomska struktura koja omogućuje konačnu razgradnju onoga što je nepotrebno ćeliji. Pored proteina, na ovaj način se mogu neutralisati različite oštećene organele, endoplazmatski retikulum, peroksizomi, lipidne kapljice, feritin i različiti zimogeni enzimi.

Šaperonima posredovana autofagija podrazumeva učešće Hsc70 i LAMP-2A (protein membrane povezan sa lizozomom 2A). U procesu makro i mikroautofagije nađeno je da šaperonima posredovana autofagija vrši degradaciju citosolnih proteina koji su prethodno bili vezani za Hsc70-specifičnu sekvencu. Od posebnog značaja je proces autofagne degradacije lipidnih kapljica, jer su u vezi sa metabolizmom lipida i značajni su u formiranju linka između poremećaja na nivou lipida i brojnih metaboličkih bolesti. Kapljice lipida u citosolu se mogu razgraditi pomoću tri autofagna puta kao što postoje tri vrste autofagije. U makrofagiji lipidnih kapljica (makrolipofagija) dolazi do zahvatanja delova lipidnih kapljica ER membranama zavisnim od proteina 1A/1B-lakog lanca 3 (LC3-II) povezanog sa mikrotubulama da bi se formirali lipoautofagozomi koji se zatim spajaju sa lizozomima u autolizozome. Tokom mikrolipofagije, proteini vezani za Ras u mozgu (RAB) olakšavaju protok lipida i proteina od lipidnih kapljica do lizozoma. Lizozomalna kisela lipaza (LAL) je jedina poznata lipaza sposobna da hidrolizuje neutralne lipide u kiselim uslovima. Nezavisno od LAL-a, autofagija posredovana šaperonom olakšava transfer posredovan Hsp70 i lizozomsko uzimanje i degradaciju perilipina zavisnu od lizozoma 2 (LAMP2) kako bi se povećala dostupnost citosolnih lipaza za lipidne kapljice. Najčešći stimulans za aktivaciju šaperonima pokrenute autofagije je nedostatak hranljivih materija (ili gladovanje), što je od posebnog značaja kada se razmatra peripartalni period kod krava. Gladovanje aktivira ovaj proces u *in vitro* i *in vivo* uslovima, a smatra se da ovaj proces zavisi od cirkulišućih ketonskih tela.

U ranijem delu teksta je izloženo da postoji velika razlika kada se radi o fiziološkim efektima intra i ekstracelularnih proteina toplotnog šoka i zbog toga je u jednom od značajnijih radova u ovoj oblasti predložena teorija bilansa čaperona, kako bi se u odnosu na dominaciju intra ili ekstracelularnih verzija ovih proteina procenio dominantno pro ili anitiinflamatorni odgovor. Masno tkivo posebno u pozitivnom energetskom bilansu i kod gojaznih jedinki je izvor koji aktivno oslobađa proinflamatorne citokine i adipokine, čime nastaje upala niskog stepena uz aktiviranje upalnih puteva zavisnih od NF-kB koji dalje dovode do: blokade intracelularnog iHsp70 i insulinske rezistencije, hroničnog oslobađanja proinflamatornog ekstracelularnog eHsp70 iz imunoloških ćelija i razvoj oksidativnog stresa, koji rezultira denaturacijom proteina. eHSP70 se povećava kao neophodan signal opasnosti za borbu protiv oksidativnog oštećenja plazme, ali kada je hronično povišen on indukuje dalju imunološku aktivaciju i proinflamatorni odgovor i aktivira toll-like receptore (TLR) i inflamatorni put koji dovodi do smanjenja aktivacije HSF-1 i na kraju do smanjenog intracelularnog iHsp70. Niži iHsp70 uzrokuje smanjenu insulinsku osetljivost, intenziviranje aktivacije i upale NF-kB i smanjeni antioksidativni, antiapoptotički i antiinflamatorni kapacitet.

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Dugoročna insulinska rezistencija određuje početak dijabetesa, upotpunjujući ovaj mehanizam pozitivne povratne informacije. Kada se odnos eHSP70/iHSP70 hronično menja u korist eHSP70, „dugme osetljivosti na insulin“ se isključuje i R vrednosti ((eHSP70)/(iHSP70)) rastu; vežbanje izaziva ekspresiju iHSP70, dok oslobađanje eHSP70 reaguje na suprotan način. R vrednosti između 0 i 1 ukazuju na antiinflamatorni status, a između 1 i 5 na optimalan status imunoinflamatornog nadzora, dok vrednosti R iznad 5 ukazuju na nepoželjan hronični proinflamatorni status.

U našem ogledu je pokazano da visoke vrednosti eHsp70 imaju dodatni nepovoljni efekat na metaboličku adaptaciju lipida i izvedenih parametara kod krava sa visokim vrednostima TNF-α. Preplitanje puteva, regulatornih mehanizama i efekata TNF-α i eHsp70 u procesima razvoja lipidoze jetre, lipolize, insulinske rezistencije i inflamacije masnog tkiva može biti razlog za nastanak dodatnih nepovoljnih efekata eHsp70, što potvrđuje pro-inflamatorno delovanje ovog čaperona u ekstracelularnoj sredini. U grupi krava gde postoji dodatni efekat eHsp70 (TNF-α+eHsp70 grupa) nađena je koncentracija BHB i NEFA koje su dovoljno visoke da mogu ispoljiti nepovoljni uticaj na sve ostale aspekte metaboličke adaptacije krava (88), što indirektno implicira potencijalni značaj eHsp70 u ukupnoj metaboličkoj adaptaciji krava u ranoj laktaciji.

LITERATURA

1. *Zhao H., Raines L.N., Huang S.C.C. Molecular Chaperones: Molecular Assembly Line Brings Metabolism and Immunity in Shape. Metabolites 2020, 10, 394. https://doi.org/10.3390/metabo10100394.*
2. *Hartl F.U., Bracher A., Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. Nature 2011, 475, 324–332.*
3. *Kim Y.E., Hipp M.S., Bracher A., Hayer-Hartl M., Hartl F.U. Molecular chaperone functions in protein folding and proteostasis. Annu. Rev. Biochem. 2013; 82, 323–355.*
4. *Vabulas R.M., Raychaudhuri S., Hayer-Hartl M., Hartl F.U. Protein folding in the cytoplasm and the heat shock response. Cold Spring Harb Perspect. Biol. 2010; 2, 12: a004390.*
5. *Fernández-Fernández M.R., Valpuesta J.M. Hsp70 chaperone: A master player in protein homeostasis. F1000Research 1000, 2018; 19.*
6. *Mahat D.B., Salamanca H.H., Duarte F.M., Danko C.G., Lis J.T. Mammalian Heat Shock Response and Mechanisms Underlying Its Genome-wide Transcriptional Regulation. Mol Cell. 2016; 62, 63-78. doi: 10.1016/j.molcel.2016.02.025. Epub 2016 Mar 24. PMID: 27052732; PMCID: PMC4826300.*
7. *Fujimoto M., Nakai A. The heat shock factor family and adaptation to proteotoxic stress. FEBS J. 2010; 277, 4112–4125.*
8. *Buzzard K.A., Giaccia A.J.; Killender M., Anderson R.L. Heat shock protein 72 modulates pathways of stress-induced apoptosis. J. Biol. Chem. 1998; 273, 17147–17153.*
9. *Ravagnan L., Gurbuxani S., Susin S.A., Maisse C., Daugas E., Zamzami N., Mak T., Jaattela M., Penninger J.M., Garrido C. et al. Heat-shock protein 70 antagonizes apoptosis-inducing factor. Nat. Cell Biol. 2001; 3, 839–843.*
10. *Muralidharan S., Ambade A., Fulham M.A., Deshpande J., Catalano D., Mandrekar P. Moderate alcohol induces stress proteins HSF1 and hsp70 and inhibits proinflammatory cytokines resulting in endotoxin tolerance. J. Immunol. 2014; 193, 1975–1987.*
11. *Jaattela M., Wissing D., Kokholm K., Kallunki T., Egeblad M. Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases. EMBO J. 1998; 17, 6124–6134.*
12. *Borges T.J., Wieten L., van Herwijnen M.J., Broere F., van der Zee R., Bonorino C., van Eden W. The anti-inflammatory mechanisms of Hsp70. Front Immunol. 2012; 3, 95. doi: 10.3389/fimmu.2012.00095. PMID: 22566973; PMCID: PMC3343630.*
13. *Kim W.S., Ghassemi Nejad J., Roh S.G., Lee H.G. Heat-Shock Proteins Gene Expression in Peripheral Blood Mononuclear Cells as an Indicator of Heat Stress in Beef Calves. Animals 2020; 10, 895. https://doi.org/10.3390/ani10050895.*
14. *Dangi S.S., Dangi S.K., Chouhan V.S., Verma M.R., Kumar P., Singh G., Sarkar M. Modulatory effect of betaine on expression dynamics of HSPs during heat stress acclimation in goat (Capra hircus). Gene 2016; 575, 543–550.*
15. *Sheikh A.A., Aggarwal A., Indu B., Aarif O. Inorganic zinc supplementation modulates heat shock and immune response in heat stressed peripheral blood mononuclear cells of periparturient dairy cows. Theriogenology 2017; 95, 75–82.*
16. *Rodrigues-Krause J.,M. Krause C. O'Hagan et al., Divergence of intracellular and extracellular HSP72 in type 2 diabetes: does fat matter? Cell Stress and Chaperones 2012; 17, 3, 293– 302.*

ISBN 978-86-7520-611-8

17. Agnew L.L., Colditz I.G. Development of a method of measuring cellular stress in cattle and sheep. Veterinary Immunology and Immunopathology 2008; 123: 197–204.
18. Asea A. Mechanisms of HSP72 release. J. Biosci 2007; 32(3): 579–584.
19. Kampinga H.H., Hageman J., Vos M.J. et al. Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chaperones 2009; 14:105–111.
20. Pittet J.F., Lee H., Morabito D., Howard M.B., Welch W.J., Mackersie R.C. Serum levels of Hsp 72 measured early after trauma correlate with survival. J Trauma 2002; 52: 611–617.
21. Molvarec A., Lilla Tamási, György Losonczy, Krisztina Madách, Zoltán Prohászka, János Rigó Jr. Circulating heat shock protein 70 (HSPA1A) in normal and pathological pregnancies. Cell Stress and Chaperones 2010; 15:237–247.
22. Ehses J.A., Meier D.T., Wueest S. et al. Toll-like receptor 2- deficient mice are protected frominsulin resistance and beta cell dysfunction induced by a high-fat diet. Diabetologia 2010; 53, 8, 1795–1806.
23. Borges T.J., Wieten L., van Herwijnen M.J. et al. The anti-inflammatory mechanisms of Hsp70. Frontiers in Immunology 2012; 3, 95.
24. Jones Q., Voegeli T.S., Li G., Chen Y., Currie R.W. Heat shock proteins protect against ischemia and inflammation through multiple mechanisms. Inflammation and Allergy-Drug Targets 2011; 10, 4, 247–259.
25. Barnes P.J. and Karin M. Nuclear factor-κB—a pivotal transcription factor in chronic inflammatory diseases. The new England Journal of Medicine 1997; 336, 15, 1066–1071.
26. Chan J.Y.H., Ou C.C., Wang L.L., Chan S.H.H. Heat shock protein 70 confers cardiovascular protection during endotoxemia via inhibition of nuclear factor-kappaB activation and inducible nitric oxide synthase expression in the rostral ventrolateral medulla. Circulation 2004; 110, 23, 3560–3566.
27. Chen H.W., Kuo H.T., Wang S.J., Lu T.S., Yang R.C. In vivo heat shock protein assembles with septic liver NF-kappaB/I-kappaB complex regulating NF-kappaB activity. Shock 2005; 24, 3, 232–238.
28. Gabai V. L., A. B. Meriin, D. D. Mosser et al. Hsp70 prevents activation of stress kinases: a novel pathway of cellular thermotolerance. Journal of Biological Chemistry 1997; 272, 29, 18033–18037.
29. Beere H.M., B.B. Wolf, K. Cain et al. Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. Nature Cell Biology 2000; 2, 8, 469–475.
30. Tsuchiya D., S. Hong, Y. Matsumori et al. Overexpression of rat heat shock protein 70 reduces neuronal injury after transient focal ischemia, transient global ischemia, or kainic acid-induced seizures. Neurosurgery 2003; 53, 5, 1179–1188.
31. Creagh E.M. and Martin S.J. Cell stress-associated caspase activation: intrinsically complex? Science's STKE 2003, 2003; 175, pe11.
32. Zheng Z., Kim J. Y., H. Ma, J. E. Lee, and Yenari M. A. Anti-inflammatory effects of the 70 kDa heat shock protein in experimental stroke. Journal of Cerebral Blood Flow and Metabolism 2008; 28, 1, 53–63.
33. Geiger P.C. and Gupte A.A. Heat shock proteins are important mediators of skeletal muscle insulin sensitivity," Exercise and Sport Sciences Reviews 2011; 39, 1, 34–42.
34. Gutierrez L.L., A. Maslinkiewicz, R. Curi, and P. I. de Bittencourt Jr. Atherosclerosis: a redox-sensitive lipid imbalance suppressible by cyclopentenone prostaglandins. Biochemical Pharmacology 2008; 75, 12, 2245–2262.
35. de Bittencourt Jr. P.I.H. and Curi R. Antiproliferative prostaglandins and the MRP/GS X pump role in cancer immunosuppression and insight into new strategies in cancer gene therapy," Biochemical Pharmacology 2001; 62, 7, 811–819.
36. Rossi A., Kapahi P., Natoli G. et al. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IκB kinase. Nature 2000; 403, 6765, 103–108.
37. Singh I.S., He J.R., S. Calderwood, and Hasday J. D. A high affinity HSF-1 binding site in the 5′-untranslated region of the murine tumor necrosis factor-alpha gene is a transcriptional repressor. The Journal of Biological Chemistry 2002; 277, 7, 4981–4988.
38. Xiao X., X. Zuo, A. A. Davis et al. HSF1 is required for extraembryonic development, postnatal growth and protection during inflammatory responses in mice. The EMBO Journal 1999; 18, 21, 5943–5952.
39. Knowlton A.A. NFkappaB, heat shock proteins, HSF-1, and inflammation. Cardiovascular Research 2006, 69, 1, 7–8.
40. Dai R., Frejtag W., B. He, Y. Zhang, and Mivechi N. F. c-JunNH2-terminal kinase targeting and phosphorylation of heat shock factor-1 suppress its transcriptional activity. Journal of Biological Chemistry 2000; 275, 24, 18210–18218.
41. Li H., X. Sun, G. Lesage et al. β-arrestin 2 regulates Tolllike receptor 4-mediated apoptotic signalling through glycogen synthase kinase-3beta. Immunology 2010; 130, 4, 556–563.
42. Ghosh S., M. J. May, and Kopp E.B. NF-κB and rel proteins: evolutionarily conserved mediators of immune responses. Annual Review of Immunology 1998; 16, 225–260.

43. Asea A., E. Kabingu, M. A. Stevenson, and Calderwood S.K. HSP70 peptidembearing and peptide-negative preparations act as chaperokines. Cell Stress Chaperones 2000; 5, 5, 425–431.
44. Asea A., M. Rehli, E. Kabingu et al. Novel signal transduction pathway utilized by extracellular HSP70. Role of toll-like receptor (TLR) 2 and TLR4. Journal of Biological Chemistry 2002; 277, 17, 15028–15034.
45. Asea A., M. Rehli, E. Kabingu et al. Novel signal transduction pathway utilized by extracellular HSP70. Role of toll-like receptor (TLR) 2 and TLR4," Journal of Biological Chemistry 2002; 277, 17, 15028–15034.
46. Kim J. J. and Sears D.D. TLR4 and insulin resistance. Gastroenterology Research and Practice 2010; 2010, ID 212563, 11 pages.
47. de Maio A. Extracellular heat shock proteins, cellular export vesicles, and the Stress Observation System: a form of communication during injury, infection, and cell damage: it is never known how far a controversial finding will go! Dedicated to Ferruccio Ritossa. Cell Stress and Chaperones 2011; 16, 3, 235–249.
48. Campisi J., T. H. Leem, and Fleshner M. Stress-induced extracellular Hsp72 is a functionally significant danger signal to the immune system. Cell Stress & Chaperones 2003; 8, 3, 272–286.
49. Njemini R., C. Demanet, and Mets T. Inflammatory status as an important determinant of heat shock protein 70 serum concentrations during aging. Biogerontology 2004; 5, 1, 31–38.
50. Bautista-Carbajal P., Duarte-Molina P., Contla-Martínez I.I., García-León M.L., Angel-Ambrocio A.H., Baltazar-López N., Wong-Chew R.M. Extracellular heat shock protein 70 is a mortality predictor in patients with septic shock and is associated with the APACHE II and SOFA scores, and the pro-inflammatory immune response. World Academy of Sciences Journal, 2021; 3, 1-8.
51. Merchant S., Korbelik M. Heat shock protein 70 is acute phase reactant: Response elicited by tumor treatment with photodynamic therapy. Cell Stress Chaperones 2011; 16, 153–162.
52. Gupta A., Cooper Z.A., Tulapurkar M.E., Potla R., Maity T., Hasday J.D., Singh I.S. Toll-like receptor agonists and febrile range hyperthermia synergize to induce heat shock protein 70 expression and extracellular release. J Biol Chem. 2013; 288, 2756–2766.
53. Asea A. Chaperokine-induced signal transduction pathways. Exerc Immunol Rev 2003; 9, 25-33.
54. Krause M., Heck T.G., Bittencourt A., Scomazzon S.P., Newsholme P., Curi R., Homem de Bittencourt P.I.Jr. The chaperone balance hypothesis: the importance of the extracellular to intracellular HSP70 ratio to inflammation-driven type 2 diabetes, the effect of exercise, and the implications for clinical management. Mediators of inflammation 2015; 249205. https://doi.org/10.1155/2015/249205.
55. Cincović M.,Belić B. Concnetration of blood Hsp70 and its relation with lipid mobilisation and ketogenesis in dairy cows during periparturient period. Contemporary agriculture 2014; 63, 1-2, 92-97.
56. Catalani E., Amadori M., Vitali A., Bernabucci U., Nardone A., Lacetera N. The Hsp72 response in peri-parturient dairy cows: relationships with metabolic and immunological parameters. Cell Stress & Chaperones 2010; 15(6): 781-790.
57. Kristensen T.N., Løvendahl P., Berg P., Loeschcke V. Hsp72 is present in plasma from Holstein-Friesian dairy cattle, and the concentration level is repeatable across days and age classes. Cell Stress & Chaperones 2004; 9(2): 143–149.
58. Molvarec A., Lilla Tamási, György Losonczy, Krisztina Madách, Zoltán Prohászka, János Rigó Jr. Circulating heat shock protein 70 (HSPA1A) in normal and pathological pregnancies. Cell Stress and Chaperones 2010; 15:237–247.
59. Molvarec A., Rigó J. Jr., Nagy B., Walentin S., Szalay J., Füst G., Karádi I., Prohászka Z. Serum heat shock protein 70 levels are decreased in normal human pregnancy. J Reprod Immunol 2007; 74:163–169.
60. Wu W.X., Derks J.B., Zhang Q., Nathanielsz P.W. Changes in heat shock protein-90 and -70 messenger ribonucleic acid in uterine tissues of the ewe in relation to parturition and regulation by estradiol and progesterone. Endocrinology 1996; 137:5685–5693.
61. Chaiworapongsa T., Erez O., Kusanovic J.P., Vaisbuch E., Mazaki-Tovi S., Gotsch F., Than N.G., Mittal P., Kim Y.M., Camacho N., Edwin S., Gomez R., Hassan S.S., Romero R. Amniotic fluid heat shock protein 70 concentration in histologic chorioamnionitis, term and preterm parturition. J Matern Fetal Neonatal Med 2008; 21:449–461.
62. Kristensen T.N., Løvendahl P., Berg P., Loeschcke V. Hsp72 is present in plasma from Holstein Friesian dairy cattle, and the concentration level is repeatable across days and age classes. Cell Stress Chaperones 2004; 9:143–149.
63. Petrović M., Cincović M.R., Belić B., Đoković R., Starič J., Ježek J. Koncentracija proteina toplotnog stresa (heat shock protein 70, hsp70) u krvnom serumu kod krava u ranoj laktaciji. 27.savetovanje veterinara Srbije, Zlatibor; 2016.
64. Febbraio M.A., Mesa J.L., Chung J., Steensberg A., Keller C., Nielsen H.B., Krustrup P., Ott P., Secher N.H., Pedersen B.K. Glucose ingestion attenuates the exercise-induced increase in circulating heat shock protein 72 and heat shock protein 60 in humans. Cell Stress Chaperones 2004; 9:390–396.

65. *Morino S., Kondo T., Sasaki K., Adachi H., Suico M.A., Sekimoto E., Matsuda T., Shuto T., Araki E., Kai H. Mild electrical stimulation with heat shock ameliorates insulin resistance via enhanced insulin signalling. PLoS ONE 2008; 3:e4068.*
66. *Catalani E., Amadori M., Vitali A., Bernabucci U., Nardone A., Lacetera N. The Hsp72 response in peri-parturient dairy cows: relationships with metabolic and immunological parameters. Cell Stress and Chaperones 2010; DOI 10.1007/s12192-010-0186-x.*
67. *Chung J., Nguyen A.K., D.C.Henstridge et al. HSP72 protects against obesity-induced insulin resistance. Proceedings of the National Academy of Sciences of the United States of America 2008; 105, 5, 1739–1744.*
68. *Kurucz I., A. Morva, A. Vaag et al. Decreased expression of heat shock protein 72 in skeletal muscle of patients with type 2 diabetes correlates with insulin resistance. Diabetes 2002; 51, 4, 1102–1109.*
69. *Atalay M., N. K. J. Oksala, D. E. Laaksonen et al. Exercise trainingmodulates heat shock protein response in diabetic rats. Journal of Applied Physiology 2004; 97, 2, 605–611.*
70. *Newsholme P. and P. I. H. de Bittencourt Jr. The fat cell senescence hypothesis: a mechanism responsible for abrogating the resolution of inflammation in chronic disease. Current Opinion in Clinical Nutrition and Metabolic Care 2014; 17, 4, 295–305.*
71. *Rodrigues Krause J., M. Krause, C. O'Hagan et al. Divergence of intracellular and extracellular HSP72 in type 2 diabetes: does fat matter? Cell Stress and Chaperones 2012; 17, 3, 293–302.*
72. *de Maio A. Extracellular heat shock proteins, cellular export vesicles, and the Stress Observation System: a form of communication during injury, infection, and cell damage: it is never known how far a controversial finding will go! Dedicated to Ferruccio Ritossa. Cell Stress and Chaperones 2011; 16, 3, 235–249.*
73. *Campisi J., T. H. Leem, and Fleshner M. Stress-induced extracellular Hsp72 is a functionally significant danger signal to the immune system. Cell Stress & Chaperones 2003; 8, 3, 272–286.*
74. *Cincović M.R. Metabolički stres krava, Monografija, Poljoprivredni fakultet-Departman za veterinarsku medicinu, Novi Sad, 2016.*
75. *McFadden J. Review: Lipid biology in the periparturient dairy cow: Contemporary perspectives. Animal, 2020; 14(S1), S165-S175. doi:10.1017/S1751731119003185.*
76. *Cincović M., Kirovski D., Vujanac I., Belić B., Djoković R. Relationship between the indexes of insulin resistance and metabolic status in dairy cows during early lactation. Acta Veterinaria 2017; 67, 57-70.*
77. *Sordillo L.M., Raphael W. Significance of Metabolic Stress, Lipid Mobilization, and Inflammation on Transition Cow Disorders. Vet Clin Food Anim 2013; 29, 267–278.*
78. *Ohtsuka H., Koiwa M., Hatsugaya A., Kudo K., Hoshi F., Itoh N., Yokota H., Okada H., Kawamura S. Relationship between serum TNF activity and insulin resistance in dairy cows affected with naturally occurring fatty liver. J. Vet. Med. Sci. 2001; 63, 1021-1025.*
79. *Contreras G.A. and Sordillo L.M. Lipid mobilization and inflammatory responses during the transition period of dairy cows. Comp. Immunol. Microbiol. Infect. Dis. 2011; 34, 281-289.*
80. *Sordillo M.L., Contrera G.A., Aitken S.L. Metabolic factors affecting the inflammatory response of periparturient dairy cows. Anim Health Res Rev 2009; 10, 53–63.*
81. *Catalani E., Amadori M., Vitali A., Bernabucci U., Nardone A., Lacetera N. The Hsp72 response in peri-parturient dairy cows: relationships with metabolic and immunological parameters. Cell Stress and Chaperones 2010; 15, 781–790. https://doi.org/10.1007/s12192-010-0186-x.*
82. *Lamy E., Jurkovich V., Rodrigues L., Geraldo A., Cachucho L., Silva F., Matos C., Capelae Silva F., Pinheiro C., Könyves L., Bakony M., Pereira A. Detection of 70 kDa heat shock protein in the saliva of dairy cows. J Dairy Res 2017; 84, 280-282.*
83. *Kristensen T.N., Løvendahl P., Berg P., Loeschcke V. Hsp72 is present in plasma from Holstein-Friesian dairy cattle, and the concentration level is repeatable across days and age classes. Cell Stress Chaperones 2004; 9, 143–149. doi:10.1379/CSC-17.1.*
84. *Archer A.E., Rogers R.S., Von Schulze A.T., Wheatley J.L., Morris E.M., McCoin C.S., Thyfault J.P., Geiger P.C. Heat shock protein 72 regulates hepatic lipid accumulation. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 2018; 315(4), R696-R707.*
85. *Shi H., Yao R., Lian S., Liu P., Liu Y., Yang Y.Y., Yang H., Li S. Regulating glycolysis, the TLR4 signal pathway and expression of RBM3 in mouse liver in response to acute cold exposure. Stress 2019; 22, 366–376.*
86. *Grabner G.F., Xie H., Schweiger M., Zechner, R. Lipolysis: cellular mechanisms for lipid mobilization from fat stores. Nature Metabolism 2021; 3, 11, 1445-1465.*
87. *Alfaro I.E., Albornoz A., Molina A., Moreno J., Cordero K., Criollo A., Budini, M. Chaperone mediated autophagy in the crosstalk of neurodegenerative diseases and metabolic disorders. Frontiers in Endocrinology 2019; 9, 778.*
88. *Cincović, R. M., Belić, B., Radojičić, B., Hristov, S., Đoković, R. Influence of lipolysis and ketogenesis to metabolic and hematological parameters in dairy cows during periparturient period. Acta veterinaria 2012; 62, 429-444.*

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Review
Pregledni rad

COST ACTION BETTER (CA20103) – AN INTERDISCIPLINARY RESEARCH NETWORK IN BIOSECURITY IN DIFFERENT ANIMAL PRODUCTION SYSTEMS

COST AKCIJA BETTER (CA20103) – INTERDISCIPLINARNA MREŽA ISTRAŽIVAČA U OBLASTI BIOSIGURNOST U RAZLIČITIM PROIZVODNIM SISTEMIMA ŽIVOTINJA

Jasna Prodanov-Radulović[a*], Telmo Nunes[b], Ilias Chantziaras[c], Branislav Kureljušić[d], Alessandra Piccirillo[e], Lena-Marie Tamminen[f], Jarkko Niemi[g], Maria Rodrigues da Costa[h], Alberto Allepuz[i]

[a] Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia
[b] Faculdade de Medicina Veterinária, Lisboa, Portugal
[c] Ghent University, Ghent, Belgium
[d] Institute of Veterinary Medicine of Serbia, Belgrade, Serbia
[e] University of Padua, Italy
[f] Swedish University of Agricultural Sciences, Sweden
[g] Natural Resources Institute Finland (Luke), Finland
[h] Scotland's Rural College - Scotland's rural college - United Kingdom
[i] Department of Animal Health and Anatomy, Universitat Autònoma de Barcelona, Spain
* Corresponding author e-mail address: Jasna Prodanov-Radulović jasna@niv.ns.ac.rs

J.P.R. https://orcid.org/0000-0002-6116-3271, T.N. https://orcid.org/0000-0002-4569-2114, I.C. https://orcid.org/0000-0001-5687-9577, B.K. https://orcid.org/0000-0003-4281-1672, A.P. https://orcid.org/0000-0002-6207-4721, L-M.T. https://orcid.org/0000-0001-6781-4533, J.N. https://orcid.org/0000-0002-9545-3509, M.R.C. https://orcid.org/0000-0003-2152-4009, A.A. https://orcid.org/0000-0003-3518-1991

ABSTRACT

Today, connecting people, researchers, research topics, ideas and projects from different countries all around the Europe is more important than ever. One of the greatest examples how research ideas can connect and integrate new research ideas is European Cooperation in Science and Technology (COST), a funding agency for research and innovation networks. The main activity is to help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This is an interdisciplinary research network that brings researchers and innovators together to investigate a topic of their choice for 4 years. COST Actions are typically made up of researchers from academia, SMEs, public institutions and other relevant organisations or interested parties. More important, COST Actions are open to all science and technology fields, including new and emerging fields and offering an inclusive, pan-European environment for individuals of all levels of seniority to grow their professional research networks. In order to explain what all the possibilities are opened by participating in a COST action, we will present an example of a successful ongoing COST Action CA20103: Biosecurity Enhanced Through Training Evaluation and Raising Awareness (BETTER) in which researchers all around Europe are actively involved. In today's world it is essential for research to be interconnected, interdisciplinary, collaborative and data-intensive. COST provides networking opportunities for researchers and innovators in order to strengthen Europe's capacity to address scientific, technological and societal challenges. By analysing the activities and presenting the results achieved so far in the COST Action BETTER, we want to highlight all the advantages and opportunities that are open to researchers in Europe by participating in the COST action.

Key words: COST Action BETTER, biosecurity, research network

KRATAK SADRŽAJ

Danas je povezivanje ljudi, istraživača, istraživačkih tema, ideja i projekata iz različitih zemalja širom Evrope značajnije nego ikada. Jedan od najboljih primera kako istraživačke ideje mogu da povežu i integrišu

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u nove je postojeći projekat Evropske saradnje u nauci i tehnologiji (COST), kojim upravlja agencija za finansiranje istraživačkih i inovacionih mreža. Glavna aktivnost je asistencija u povezivanju istraživačkih inicijativa širom Evrope i omogućavanje naučnicima da razvijaju svoje ideje tako što će ih podeliti i sa kolegama iz drugih država. Zapravo to je interdisciplinarna istraživačka mreža koja okuplja istraživače i inovatore da zajednički istražuju temu po svom izboru tokom 4 godine. COST Akcije obično obuhvataju učesnike, istraživače iz akademske zajednice, malih i srednjih preduzeća, javnih institucija i drugih relevantnih organizacija ili zainteresovanih strana. Što je još važnije, COST Akcije su otvorene za sve oblasti nauke i tehnologije, uključujući tu i nova polja I omogućavaju inkluzivno, panevropsko okruženje za pojedince svih nivoa radnog iskustva kako bi i sami razvili svoje profesionalne istraživačke mreže. U cilju da se prezentuju sve mogućnosti koje se otvaraju učešćem u jednoj COST akciji, u radu je analiziran primer jedne uspešne COST CA20103 koja je u toku po nazivom: Biosigurnost poboljšana kroz obuku, evaluaciju i podizanje svesti (BETTER) u koju su aktivno uključeni istraživači širom Evrope. U današnje vreme je od suštinske važnosti da istraživanja budu međusobno povezana, interdisciplinarna, kolaborativna i sa brojnim podacima. COST akcija pruža mogućnosti umrežavanja istraživačima i inovatorima u Evropi u cilju jačanja kapaciteta i mogućnostima napredka u naučnim, tehnološkim i društvenim izazovima. Analizom aktivnosti i predstavljanjem do sada postignutih rezultata u COST Action BETTER, istaknute su sve prednosti i mogućnosti koje se otvaraju istraživačima u Evropi učešćem u COST akciji.

Ključne reči: COST Akcija BETTER, biosigurnost, istraživačka mreža

INTRODUCTION TO THE EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY ACTIONS

One of the greatest examples how research ideas can connect and integrate new research ideas is European Cooperation in Science and Technology (COST), a funding agency for research and innovation networks. The main activity is to connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. A COST Action represent an interdisciplinary research network that brings researchers and innovators together to investigate a topic of their choice for 4 years. The COST projects are made up of researchers from academia, small and medium-sized enterprises (SMEs), public institutions and other relevant organisations or interested parties. They are open to all science and technology fields, offering an inclusive and pan-European environment for individuals of all levels of seniority to grow their professional research networks and boost their careers. The COST Actions help to advance knowledge and strengthen the research and development sector, by creating networking opportunities for researchers to meet and discuss ideas, addressing the complex problems in a targeted way, across a large geographical area. All information's how to apply can be found at the website of European Cooperation in Science and Technology (1).

In order to present all the possibilities that are opened by participating in a COST action, the ongoing COST action CA20103: Biosecurity Enhanced Through Training Evaluation and Raising Awareness (BETTER) will be shortly presented in which participants, researchers all around Europe are actively involved (2).

BACKGROUND OF THE COST ACTION BETTER

Nowadays, health and welfare, safety and sustainability are crucial elements for ensuring the future of livestock production. Biosecurity aims to prevent the introduction and spread of pathogens within and between farms and, consequently, results in better welfare, increased food safety and better sustainability. In addition, biosecurity measures act as a barrier against the periodic emergence of transboundary diseases. The present Action is supported by the growing interest for biosecurity but also based on the increased number of research projects on these aspects. The most importantly, better communication and coordination mechanism is needed between the different groups in order to give a common voice to those in the field (i.e., farmers, veterinarians, traders, decision makers and the general population) (3,4).

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DESCRIPTION OF THE COST ACTION BETTER

Biosecurity is of paramount importance to prevent the introduction and spread of pathogens and, consequently, to preserve the health of farmed animals. Healthier animals result in better animal welfare, better sustainability of animal production systems and less antimicrobial use. Despite these benefits, biosecurity is limited by different factors: lack of knowledge on ways for improvement; shortage of adequate ways to enhance communication; diversity of methodologies to assess and measure the implementation of biosecurity measures and their cost-effectiveness and a low number of trained professionals (2). To approach these challenges, the Action will evaluate how biosecurity is currently used and will use participative approaches to understand motivators and barriers for biosecurity implementation. Knowledge generated through them will act as the baseline upon which to develop adequate communication and training on biosecurity. The Action will also perform a comparison of existing methods used to evaluate biosecurity. Exploiting these tools will promote the development of tailored options in farms based on the evaluation of their risks, on the feasibility of selected biosecurity measures and on their economic benefits. Moreover, the Action will identify training needs through the evaluation of existing training materials and will develop new courses, increasing therefore the number of trained professionals. Finally, the Action will recommend priority research areas for future biosecurity improvement in animal production systems (2,3,4).

OBJECTIVES AND THE STRUCTURE OF THE COST ACTION BETTER

The overall aim of the Action is to reduce the risk of infectious disease introduction and spread by improving the implementation of biosecurity measures in animal production systems. The COST Action BETTER is structured in four working groups (WG): WG1-Mapping biosecurity measures applied on farms and transport across Europe; WG2-Scaling-up the knowledge and experience of stakeholders and of the general public; WG3-Methods for evaluation of biosecurity and benefits of its implementation; WG4-Training and dissemination (2).

The first working group (WG1) is addressing how biosecurity measures are applied in Europe and neighbouring countries, and will identify ways for improvement, especially in production systems where there is a lower level of implementation or in settings where biosecurity is more challenging to carry out. This working group will address the challenge of understanding how biosecurity measures are applied in the different cattle, pig and poultry production systems across Europe in order to identify existing knowledge gaps and ways for improvement. The first objective of this working group is to map the implementation of biosecurity measures in livestock holdings and in animal transport. In order to achieve tasks, in the first year of COST Action in WG1 three subgroups had been established and they developed a template to map biosecurity practices across Europe in each species (i.e., cattle, pigs, and poultry production). A data collection tool has been created to capture biosecurity practices applied in animal production systems across Europe. The created database is related to biosecurity measures (mandatory by law and by other than law) and their implementation rate. Today, data collection on biosecurity practices applied in animal production systems has been completed in the previous period and collected information are analysed (5).Moreover, in the second year of the project a new subgroup in small ruminants has been created. The current WG1 activity includes a new survey which intends to capture the different existing definitions about small farms and which biosecurity measures are mandatory. The other survey will target field vets and animal health technicians, to identify attitude towards biosecurity in small farms animal production and try to identify the areas that they see as feasible to improve.

The second working group (WG2) is addressing the challenge of the Action which is to scale-up the knowledge and experience of end-users of biosecurity and of the general society. This is highly relevant as any training or communication strategies for end-users should be based not only on science but also on the understanding of why stakeholders behave the way they do. Currently, in this working group collected data from the survey for consumers to assess their perception of biosecurity and the role they could play in disease spread has been analysed.

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Also, data extraction on the literature reviews on the social, economic, and psychological factors affecting the decision-making to implement biosecurity measures by farmers, veterinarians and traders is progressing. Participative actions to evaluate how biosecurity is being communicated have been initiated (2).

The third working group (WG3) in COST Action is focusing on identifying the existing methods used to evaluate biosecurity. At present, methodologies are diverse and have not been compared comprehensively. Such methods can be useful as educational tools and for developing skills in risk-based prioritisation as well as to provide quantitative goals and benchmarks which can be used to position the farm with respect to others. The challenge of WG3 will be to address the heterogeneity of the existing methods to evaluate and promote biosecurity (6). The WG3 has developed an online survey to identify and describe methods currently used in different countries to assess biosecurity (7). Moreover, WG3 has developed guidelines to conduct a literature review on methods to assess biosecurity and to demonstrate the benefits of implementing biosecurity. At the moment, data collected through the online survey about methods currently used in different countries to assess biosecurity has been analysed. The next task related to evaluate the effectiveness of improvement biosecurity programs has already begun.

The fourth working group (WG4) aims to increase the number of professionals, such as veterinarians and farmers, trained in biosecurity and to develop guidelines and good practices for evidence-based effective communicative strategies for different stakeholders. This working group will address the challenge of increasing the number of professionals trained in biosecurity and the development of communicative strategies with a high impact amongst the different stakeholders. The survey on the identification of training needs on biosecurity among different stakeholders has been done and data analysed (8). Participative actions to evaluate training needs are under development.

Common task for all WGs is to create a sustainable network for the promotion of research, education and application of biosecurity measures in European countries. Through the different online and face-to-face meetings the network is getting engaged and is being consolidated. Also important task for all WGs is to improve the communication and information sources related to biosecurity aimed at veterinarians, farm advisors, farmers' associations and official authorities. Data collected through the project (i.e., biosecurity measures that are being implemented, how they are implemented, etc.) will be made available to veterinarians, farm advisors, etc., once data collection and analysis has been finished.

THE TEAM OF THE COST ACTION BETTER

The present Action involves a multidisciplinary team (e.g., economists, epidemiologists, evidence synthesis experts, infectious diseases and animal production experts, Sociologists, psychologists, and communication experts, among others) encompassing the expertise needed to solve the identified challenges. The number of people involved in the Action together with their different backgrounds and the wide geographic distribution provides an excellent opportunity to achieve the objectives of the Action as they will enable the contact with stakeholders across an extensive area. At present (March 2024), the Action is composed of 347 participants from 47 different countries including non-EU countries (e.g. Australia, Argentina, Canada and New Zealand). In addition, stakeholders from government bodies, the industry or international organizations such as FAO and OIE have also joined the Action.

ACTIVITIES AND MEETINGS OF THE COST ACTION BETTER

The COST Action BETTER is organizing the several activities that are running parallel to the tasks planned in the working groups. First, COST Action BETTER organise two face-to-face WGs meetings every year in order to ensure networking and facilitate work progress of the different WGs. The different WGs had some time to discuss their current activities. During these meetings, also specific workshops are organised in different working groups for in-depth discussion of some topics. Additionally, all participants can submit the abstract and have the flash talks and/or present their work in poster session (2). Indeed, in all previous meetings, posters were displayed based on participants contributions and some number of them were selected and presented as a flash talk. In between face-to-face meetings, the WG leaders are

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organising on-line meetings for all registered participants in different WGs.

Management committee (MC) meetings (with the exception of the first kick-off meeting), are organized on-line after COST Action meetings, in order to optimize resources. Beside WGs and MC meetings, the members of the Core group have on-line meetings monthly in order to monitor progress of the different planned activities and to promote new ideas and activities. They are also in charge of organizing the Short-Term Scientific Missions (STSM/VM), the different Action WG Meetings and conferences. Short-Term Scientific Missions are organized based on the working needs of each of the working groups. STSM consists of a visit to a host organization located in a different country than the country of affiliation by a researcher or innovator to complete a specific work or task in a determined time. VM consists of collaboration in a virtual setting among researchers or innovators (they work from home country), to achieve determined tasks, exchange knowledge, learn new techniques, etc. Both aim to strength the existing networks and foster collaborations among institutions. COST Action BETTER Open Calls also include Conference dissemination grants which include financial support to Action participant for their participation in high-level conferences. Moreover, inclusiveness target country (ITC) Conference Grants consist of financial support for Young Researchers and Innovators (<40 years) affiliated in an Inclusiveness Target Country / Near Neighbour Country for their participation in high-level conferences (2). All the working groups are participating in the organization of different training courses, one in each region of Europe (i.e., west and east). Therefore, the Action aims to teach at least two biosecurity courses at the end of the Action. In the final year of the COST Action the Final workshop is planned and all WGs will participate to identify main biosecurity gaps and brainstorming on ways for improvement.

A webinar series has been initiated to promote communication and information sources (9). In the BETTER webpage a repository section was created in which presentations from the different meetings are stored, which are accessible to any interested person (2).

THE PROGRESS OF COST ACTION BETTER DURING ITS FIRST TWO YEARS

In the first two years COST Action BETTER have been able to characterize how biosecurity is being applied in farms in different countries and which measures are compulsory by law and which are not. The collected data will contribute to identify which are the main gaps and areas in which more research is needed. Moreover, the perception of the general public in the role that they can have in disease spread is being evaluated and different barriers and motivators are being compiled. Indeed, 88 different methods used to evaluate biosecurity in different countries have been identified and are being characterized. The findings from this study will aid in identifying deficiencies in biosecurity assessment methods for certain animal species, highlighting both commonalities and disparities among these methods. Moreover, countries could leverage these results to initiate their own evaluations or to re-evaluate their existing methodologies. Moreover, exiting training and research projects in biosecurity are being compiled, which will also serve as the basis to identify training gaps and recommend further trainings on biosecurity.

CONCLUSIONS

Currently the COST Action BETTER is approaching to the end of the third grant period, and have been able to achieve most of goals planned for this period. The visibility of the BETTER has significantly increased through the participation in several conferences and relevant meetings across Europe and we are beginning to prepare the first drafts of several papers.

In today's world it is essential for research to be interconnected, interdisciplinary, collaborative and data-intensive. COST provides networking opportunities for researchers and innovators in order to strengthen Europe's capacity to address scientific, technological and societal challenges (1). By analysing the activities and presenting the results achieved so far in the COST Action BETTER, we want to highlight all the advantages and opportunities that are open to researchers in Europe by participating in the COST action.

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LITERATURE

1. COST (European Cooperation in Science and Technology) available at: https://www.cost.eu/cost-actions/what-are-cost-actions/ Accessed 01-04-2024.
2. BETTER - COST ACTION CA20103 Biosecurity Enhanced Through Training Evaluation and Raising Awareness available at: https://better-biosecurity.eu/ Accessed 01-04-2024.
3. Allepuz A., Kjosevski M., Beber C. L., Tamminen L.-M., Saegerman C., Hilal-Yavuz H., Prodanov-Radulović J., Niine T., Rodrigues C.M., Ciaravino G., Chantziras I.: A new COST Action to improve biosecurity in livestock. Book of abstracts,14thAnnual Meeting of EPIZONE, 18-20.05.2022., Barcelona, Spain, 2022, p.227.
4. De Nardi M., Chantziaras I. and Allepuz A.: Biosecurity Enhanced Through Training Evaluation and Raising Awareness (BETTER): a Cost Action project to improve biosecurity implementation at farm level. ECVPH Annual Scientific Conference, Athens, Greece 28-30 September 2022, p.40.
5. Prodanov-Radulović J., Nunes T., Chantziaras I., Kureljušić B., Piccirillo A., Tilli G., Allepuz A.: Mapping biosecurity measures applied on different animal production systems across Europe – Progress of COST Action BETTER. Book of abstracts, 15th EPIZONE Annual Meeting "New Perspectives for the New Era", Novi Sad, Serbia, April 26-28, 2023, p.158.
6. Duarte F., Ciaravino G., Tamminen L.-M., Kjosevski M., Díaz I., Nunes T., Gomes C., Prodanov-Radulović J., Vodica A., Gecaj R., Staric J., Allepuz A.: Methods used to assess biosecurity. Book of Abstracts, The Annual General Meeting of the COST action BETTER, 7-8 February 2023, Ghent, Belgium, p.8.
7. Duarte, F., Ciaravino G., Valeria L., Svennesen L., Viltrop A., Toppari I., Delpont M., Ózsvári L., Correia-Gomes C., Piccirillo A., Gecaj R., van den Borne B., Kjosevski M., Żbikowski A., Nunes T., Prodanov-Radulovic J., Veren L., Tamminen L.M., De Nardi M., Nedosekov V., Desvars-Larrive, Allepuz, A.: Characterization of methods used to assess biosecurity on animal production farms in different countries. Book of Abstracts, The Annual General Meeting of the COST action BETTER, 6-7 February 2024 Padova, Italy, p.25.
8. Saegerman C., Parisi G., Niemi J., Humblet M.-F., Ron-Román J., Souley Kouato B., Allepuz A., Porphyre V., Rodrigues da Costa M., Renault V. Evaluation Survey on Agreement with Existing Definitions of Biosecurity with a Focus on Livestock. Animals 2023, 13, 1518. https://doi.org/10.3390/ani13091518
9. Webinars available at: https://better-biosecurity.eu/webinars/ Accessed 01-04-2024.

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Review
Pregledni rad

SIGNIFICANCE OF CLINICAL MASTITIS IN DAIRY COWS

ZNAČAJ KLINIČKOG MASTITISA KOD MLEČNIH KRAVA

Miodrag Radinović[a], Zorana Kovačević[a], Ivana Davidov[a], Mihajlo Erdeljan[a], Marija Pajić[a], Jovan Stanojević[a], Ramiz Ćutuk[b]

[a]University of Novi Sad, Faculty of Agriculture, Department of Veterinary medicine, Serbia
[b]University of Sarajevo, Faculty of Veterinary medicine, BiH

M.R. https://orcid.org/0000-0002-4390-4670, Z.K. https://orcid.org/0000-0002-0133-872X, I.D. https://orcid.org/0000-0003-2630-3061, M.E. https://orcid.org/0000-0002-4566-7584, M.P. https://orcid.org/0000-0001-9756-2461, J.S. https://orcid.org/0009-0000-3328-6963, R.Ć. https://orcid.org/0000-0002-1058-8755

ABSTACT

Mastitis represents one of the most significant health disorders in dairy cow herds. They can produce significant direct and indirect losses. In relation to the intensity of the changes that are present, mastitis can be marked as subclinical or clinical. Clinical forms of mastitis are accompanied by clear changes in the mammary gland tissue and secretion of the mammary gland and changes in the general condition of the animal. Often, if there is no adequate and timely therapy, they can have a fatal outcome. Because of this, it is necessary to approach the procedure of diagnosis and treatment of severe clinical forms of mastitis with special attention. This includes program of mastitis control with focus on clinical changes and emphasis on urgent therapy.

Keywords: cow, clinical mastitis, treatment

SAŽETAK

Mastitis predstavlja jedan od najznačajnijih zdravstvenih poremećaja u stadima muznih krava. Oni mogu proizvesti značajne direktne i indirektne gubitke. U odnosu na intenzitet prisutnih promena, mastitis se može označiti kao subklinički ili klinički. Kliničke forme mastitisa prate jasne promene u tkivu mlečne žlezde i sekreciji mlečne žlezde i promenama opšteg stanja životinje. Često, ako nema adekvatne i blagovremene terapije, mogu imati fatalan ishod. Zbog toga je potrebno sa posebnom pažnjom pristupiti postupku dijagnostike i lečenja teških kliničkih oblika mastitisa. Ovo uključuje program kontrole mastitisa sa fokusom na kliničke promene i naglaskom na hitnu terapiju.

Ključne reči: krave, klinički mastitis, terapija

INTRODUCTION

Mastitis occurs as a result of the penetration of microorganisms into the mammary gland, most often bacteria, and the establishment of an intramammary infection and, consequently, an inflammatory reaction. In addition to microorganisms, the etiology of mastitis includes the action of other harmful factors, physical, mechanical and chemical, which can freeze the tissue of the mammary gland and cause an inflammatory reaction. Therefore, mastitis is defined as inflammation of the mammary gland, which, in addition to physical and chemical changes in the milk, is accompanied by pathological changes in the gland itself, as well as changes in the general health status (8). The form of clinical manifestation defines two types of mastitis, clinical and subclinical (12). In clinical mastitis, the signs of inflammation on the mammary gland are clearly manifested in the form of redness, swelling, soreness, temper, and it is often accompanied by a change in the general condition of the animal through an increase in body temperature, lethargy and loss of appetite (9). In addition to this, in clinical mastitis there are clearly

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visible changes in the secretion of the mammary gland, both in appearance and in quantity, until the complete cessation of secretion. Subclinical mastitis is a form of the disease in which there are no noticeable clinical symptoms on the mammary gland or changes in the general condition. What is noticeable is the decrease in the amount of milk, and laboratory analysis can prove changes in the composition of milk (15,16). The most obvious indicator is a change in the number of somatic cells in the milk. Both forms of mastitis cause significant economic losses and therefore the importance of mastitis prevention is highlighted (8, 10). The existence and application of a mastitis control program on dairy cow farms has shown a good effect, especially in order to control specific udder pathogens (14). Nevertheless, the problem of mastitis still requires a wide application of antibiotics in order to treat and prevent mastitis. According to data from the literature, mastitis is the most common indication for the use of antibiotics in cattle (15, 18). This represents an additional problem because it promotes the development of resistance in bacterial causes of mastitis and increases the risk of antibiotic residues and resistant strains of microorganisms entering the human food chain. In addition to this, the economic aspect is also important in terms of the price of antibiotic therapy and the price of discarded milk due to the duration of the withdrawal period (11). What must certainly not be neglected is the issue of the welfare of dairy animals. Mastitis represents a very stressful and painful condition for the animal, milking in the case of clinical mastitis is very painful but necessary to promote healing (11). Etiology of mastitis About 140 species, subspecies and serotypes of microorganisms that can cause inflammation of the mammary gland were isolated from the mammary gland of cows. The causative agents of mastitis can be divided into infectious agents and opportunistic or environmental pathogens. Further classification can be divided into agents of high pathogenicity (those that cause the clinical form of mastitis) and agents of low pathogenicity (those that cause a subclinical form of mastitis or less often lead to a clinical form of mastitis (4). Infectious causative agents of cow mastitis are numerous. The most important species are *Staphylococcus aureus, Streptococcus agalactiae and Mycoplasma bovis* (4). The usual source of these pathogens is the infected mammary gland of cows that already suffer from mastitis. In addition to this source of infection, a very significant source of infection are the hands of milkers, on which *Staphylococcus aureus* can be found. These pathogens spread from infected neighborhoods to non-infected ones. Environmental pathogens are those species that are normally found in the animal's environment and on the skin of the mammary gland, but do not cause infection (19). The frequency of infection caused by these pathogens is increasing, especially in herds where the most important pathogens, the causative agents of mastitis, are controlled. The most important pathogens from this group are coagulase-negative staphylococci, which cause infections of the mammary gland through the ascending route. Pathogens of environmental origin that can lead to mastitis are numerous. For easier systematization, they are divided into three main groups. These are coliform microorganisms that include *Escherichia coli*, *Klebsiella spp*. and *Enterobacter spp*. then bacteria from the genus *Streptococcus (Streptococcus uberis*, *Streptococcus dysgalactiae* which have a higher prevalence and *Streptococcus equinis* or *Streptococcus bovis* which have a lower prevalence) and Corynebacterium *pyogenes* (4). Risk factors Risk factors are very important for the occurrence of mastitis. They allow infectious agents to quickly and easily lead to inflammation of the mammary gland. They can be related to the animal, then to milk production, and finally to the quality of accommodation and hygiene of the mammary gland itself. The age of animals and the number of lactations increase the risk of mastitis, and the maximum is reached at 7 years. Surveys on the prevalence of intramammary infection in heifers, a few days before the first parturition, show that 4% are infected (13). The prevalence of new infections ranges between 29% and 75% of affected quarters before calving and from 12% to over 57% of infected quarters (6). The stage of lactation also plays a significant role in the development of mastitis. Most of the newly infected occur in the early stage of drying and in the first two months of lactation. In heifers, a high prevalence of intramammary infection occurs in the last trimester of pregnancy and a few days after parturition, and milk production visibly decreases. In heifers, infection in the peripartum period is mainly related to pathogens of lesser pathogenicity, but some studies also show infections with pathogens of high pathogenicity (6). The relationship between the season and occurrence of mastitis depends on geographical and climatic conditions. In subtropical

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and tropical regions, mastitis occurs more often in winter or spring, most likely due to high humidity. In temperate regions and controlled conditions, the period during which mastitis most often occurs is summer, probably due to the higher temperature in the facility, which facilitates and contributes to the growth and development of bacteria in the mat (4,5). The morphology and structure of the teat can influence the occurrence of mastitis in cows. The very end of the teat represents the first barrier that prevents the penetration of bacteria (19). Damage to this part can lead to intramammary infection. The thickness of the teat can help assess the condition of the tissue. Depending on the characteristics, the milking machine can cause the teat thickness to decrease or increase after milking. There is an association between hyperkeratosis and a higher microbial burden of the mammary gland, especially for the two common environmental pathogens Escherichia coli and Streptococcus uberis, but not for Staphylococcus aureus (13, 17). Nipple hyperkeratosis is associated with an increased risk of clinical mastitis (2). Udder hygiene is also a very important risk factor for mastitis. Dirty udders are associated with an increased prevalence of intramammary infections caused by pathogens from the external environment (3).

Colimastitis

Colimastitis represents a very severe form of parenchymatous mastitis caused by pathogenic coliform microorganisms. This form of mastitis occurs in some animals in the period of high lactation and develops very quickly. It is also called mastitis paralytica because the affected animals often cannot get up, they usually lie down for up to 48 hours. The affected quarter swells up a lot, dries up and becomes painful to the touch. The infection is not directly transmitted from cow to cow. The causative agents of colimastitis are enterobacteria, and the primary causative agent *is E.coli*. Therefore, the term colimastitis is generally used for all udder inflammations caused by enterobacteria. Apart from *E.coli*, *Klebsiella pneumoniae*, *Enterobacter agglomerans, Enterobacter cloacae, Citrobacter diversus*, *Serratia marcescens*, *Serratia odorifera* are also important causative agents. The natural intestinal flora includes a large number of representatives of the Enterobacteriaceae family, especially Escherichia. E. coli, as the causative agent, is represented in the highest percentage compared to other gram-negative bacteria (1,2,4), they claim that it accounts for 66.0%.

Gangrenous mastitis

It represents severe clinical mastitis with an often fatal outcome. (6,9, 20). Regressive processes that spread rapidly, as well as circulation and permeability disorders, are in the first place in the development of diseases. Interalveolar inflammatory edema, high-grade hyperemia or perialveolar capillary and diapedesic hemorrhages lead to softening of the parenchyma of the mammary gland. The blood vessels of the intraalveolar and interlobular tissue also show fibrinoid wall necrosis and partial or total blockages due to fibrin thrombi. Edema formation, fibrin deposition and lymphatic vessel thrombosis were found in the interlobular cavity. The clinical findings on the udder indicate a change in the color of the tissue to dark purple, the tissue is cold to the touch and the secretion is bloody. The etiology is complex, primarily Staphylococcus *aureus, Escherichia coli, Clostridium perfringens*, but also other causative agents can lead to gangrenous changes on the udder.

Mycoplasma mastitis

Mycoplasma bovis, previously designated as *Mycoplasma agalactiae* var. *bovis* and *Mycoplasma bovimastidis* is considered the most important causative agent of mycoplasma mastitis of cattle in the countries of Western Europe. These mastitis in the herd are enzootic - often 20% or more cows in the herd are affected - and manifest in a severe clinical form (4). In mastitis caused by Mycoplasma bovis, the most severe forms of clinical inflammation of the udder occur. Animals in all stages of lactation, including dry cows, are affected. The frequency and intensity of mastitis can be observed in the prenatal and postpartum period. The sudden, rapid onset of mastitis is characteristic, associated with a rapid decrease in the amount of milk, up to 10% between two milkings, and after 3-5 days it is reduced to only a few milliliters (6). The affected quarters are initially swollen, but there is no local pain or increased warmth of the quarters. The disease is often progressive and after about 3-4 weeks there is atrophy of the udder parenchyma.

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REFERENCE

1. *Bradley A.J., Breen J.E., Payne B., White V., Green M.J. An investigation of the efficacy of a polyvalent mastitis vaccine using different vaccination regimens under field conditions in the United Kingdom. J. Dairy Sci. 2015, 98:1706–1720.*
2. *Breen J.E., Green J.M., Bradley J.A. Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom, Journal of Dairy Science 2009, 92:2551.*
3. *Compton C.W.R., Heuer C., Parker K., McDougall S. Risk Factors for Peripartum Mastitis in Pasture-Grazed Dairy Heifers, Journal of Dairy Science 2007, 90:4171.*
4. *Constable D.P., Hinchcliff W.K., Done H.S., Grünberg W. 2017. Veterinary medicine, a textbook of the diseases of cattle, horses, sheep, pigs and goats, 11th edition, St. Louis, Missouri.*
5. *de Boyer des Roches A., Faure M., Lussert A., Herry V., Rainard P., Durand D., Foucras G. Behavioral and patho-physiological response as possible signs of pain in dairy cows during Escherichia coli mastitis: A pilot study. J Dairy Sci 2017, 100(10):8385-8397.*
6. *de Vliegher S., Fox K.L., Piepers S., McDougall S., Barkema W.H. Invited review: Mastitis in dairy heifers: Nature of the disease, potential impact, prevention, and control, Journal of Dairy Science 2012, 95, 3.*
7. *De Vliegher S., Ohnstad I., Piepers S. Management and prevention of mastitis: A multifactorial approach with a focus on milking, bedding and data-management, Journal of Integrative Agriculture 2018, 17, 6, 1214-1233.*
8. *Dego O.K. Bovine mastitis:Part I, open access peer-reviewed chapter, 2020.*
9. *Harmon R.J. Physiology of mastitis and factors affecting somatic cell counts. Journal of Dairy Science 1994, 77, 2103– 2112.*
10. *Hogan S.J., Smith L.K., Todhunter A.D., Schoenberger S.P. Efficacy of a Barrier Teat Dip Containing 0.55% Chlorhexidine for Prevention of Bovine Mastitis. Journal of Dairy Science 1995,78, 2502-2506.*
11. *Lam, T.J.G.M., Olde Riekerink R.G.M., van Engelen E., Hage J.J. Towards a proactive approach of antibiotic use in cattle practice. In: Proceedings of the 6th European Congress of Bovine Health Management. Société Belge Francophone de Buiatrie & Vlaamse vereniging voor Buiatrie, Belgium. pp. 17–26, 2011.*
12. *Ohnstad I. Liner mapping and teat health. Proceedings of the British Mastitis Conference. Institute of Animal Health, United Kingdom, Worcester. pp. 7–14, 2012.*
13. *Paduch J.H., Mohr E., Krömker V. The association between teat end hyperkeratosis and teat canal microbial load in lactating dairy cattle. Vet Microbiology 2012, 158:353.*
14. *Radinović M., Boboš S., Davidov I., Pajić M. Comparative analysis of somatic cell count in milk samples during winter and summer period. Middle European Buiatric Congress, Pula, 2011.*
15. *Radinović M., Boboš S., Mihajlović-Ukropina M., Pajić M. Uticaj patogena prvog reda na higijensku ispravnost mleka. Contemporary Agriculture 2008, 57, 3-4, 39-45.*
16. *Radinović M., Boboš S., Pajić M., Galfi A. Uticaj uzročnika mastitisa na higijensku ispravnost mleka, 25. Savetovanje veterinara Srbije. 11-14 Septembar, Zlatibor, 2014.*
17. *Radinović M., Boboš S., Pajić M., Galfi A., Davidov I. Mere biosigurnosti i pojava mastitisa u intenzivnoj proizvodnji mleka. 17. Savetovanje doktora veterinarske medicine Republike Srpske. 27 Jun, Teslić, 2012.*
18. *Radinović M., Boboš S., Pajić M., Mihajlović-Ukropina M., Kujača V. Findings of Streptococcus agalactiae in cow udder, proposal of control and eradication. Contemporary Agriculture 2009, 58, 1-2, 136-140.*
19. *Radinović M., Davidov I., Erdeljan M., Pajić M., Boboš S. Effect of udder infection with various microorganisms on milk production in cows. 23. International Symposium "New Technologies in Contemporary Animal Production". 19-21 Jun. Novi Sad, 2013.*
20. *Radinović M., Pajić M., Boboš S. Nalaz Staphylococcus aureus u vimenu krava sa poremećenom sekrecijom. 8. Kongres veterinara Srbije. 15-19 Septembar, Beograd, 2009.*

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Article
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CHANGES OF TOTAL PROTEINS DURING MATURATION PERIOD OF SJENICA CHEESE

PROMENE UKUPNIH PROTEINA TOKOM ZRENJA SJENIČKOG SIRA

Željko Savić[a], Aleksandar Čukić[a*], Ljiljana Andjušić[a] and Božidar Milošević[a]

[a]Faculty of Agriculture, University of Priština in Kosovska Mitrovica, Kopaonička bb, 38219 Lešak, Serbia
*Corresponding author: aleksandar.cukic@pr.ac.rs

Ž.S. https://orcid.org/0009-0006-5932-3551, A.Č. https://orcid.org/0000-0001-9063-3431, Lj.A. https://orcid.org/0000-0002-0054-1128, B.M. https://orcid.org/0000-0003-4381-0276

ABSTRACT

Proteins are one of the most important milk quality parameters and the course of coagulation, composition and technological properties of cheese curd depend on them. Proteins form the basis of the structure of curds and cheese in which milk fat is incorporated, and represent the substrate for enzymes during the ripening of Sjenica cheese. Sjenica cheese is originally produced on the Sjenica-Pester plateau, from whole, fresh, sheep and cow milk, and belongs to the group of white cheeses in brine. Considering the importance of proteins, this research aimed to determine the protein content after cheese making, and to monitor changes after 30, 60, 120, 180 days of ripening, and the extent of degradation using electrophoresis. The results of the research showed that the total protein content was the highest in Sjenica cheese after 30 days of ripening (15.54%), and in Sjenica type cheese after 60 days of ripening (16.55%). After 30, i.e. 60, 120, 180 days of ripening, there is a slight decrease in total protein in both cheeses, as a result of the transfer of soluble nitrogen from the cheese to the ripening brine. After 180 days, the total protein content was 12.78% for Sjenicki and 13.92% for Sjenicki type cheese. Electrophoretic studies showed that protein degradation occurred mostly during the first 60 days of ripening, after that the protein fractions appear in traces as diffuse zones.

Keywords: Sjenica cheese, total proteins, proteolysis, ripening, electrophoresis.

SAŽETAK

Proteini su jedan od najvažnijih parametara kvaliteta mleka, od kojih zavisi tok koagulacije, sastav i tehnološke osobine gruša. Proteini čine osnovu strukture gruša i sira u koje je inkorporirana mlečna mast, a predstavljaju supstrat za enzime tokom zrenja Sjeničkog sira. Sjenički sir izvorno se proizvodi na Sjeničko-pešterskoj visoravni, od punomasnog, svežeg, ovčijeg i kravljeg mleka, a pripada grupi belih sireva u salamuri. Obzirom na značaj proteina, istraživanja su imla za cilj da se utvrdi sadržaj proteina nakon izrade sireva, a zatim pratiti promene tokom zrenja nakon 30, 60, 120, 180 dana, a elektroforezom i obim razgradnje. Rezultati istraživanja su pokazali da je sadržaj ukupnih proteina imao maksimum kod Sjeničkog sira nakon 30 dana zrenja(15,54%), a kod sira u tipu Sjeničkog posle 60 dana zrenja(16,55%). Nakon 30 odnosno 60, 120, 180 dana zrenja beleži se blago smanjenje ukupnih proteina kod oba sira, kao rezultat prelaska rastvorljivog azota iz sira u salamuru za zrenje. Nakon 180 dana sadržaj ukupnih proteina iznosio je 12,78% za Sjenički i 13,92% za sir u tipu Sjeničkog. Elektroforetska ispitivanja su pokazala da je do razgradnje proteina u najvećoj meri došlo tokom prvih 60 dana zrenja, nakon čega se proteinske frakcije javljaju u tragovima kao difuzne zone.

Ključne reči: Sjenički sir, ukupni proteini, proteoliza, zrenje, elektroforeza.

1. INTRODUCTION

For majority types of cheeses, the greatest importance of all milk components in the production belongs to casein. Technological process of cheese production is based on the specific properties of casein. The ability to coagulate and to form milk curd stands out, as well as syneretic property of

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formed curd, and the tendency to proteolysis, under the influence of proteolytic enzymes found in cheese. The specific properties of casein, that are needed for cheese production, come from its structure and micellar form (1).

Proteins build a protein matrix that forms the basis of the cheese structure, in which a large part of the mineral complex of the cheese is incorporated, so they are the bearers of the firmness and elasticity of the cheese and the greatest potential for changes in the cheese dough during ripening under the influence of enzymes. All enzymes involved in the ripening process are in the watery phase of the cheese. As cheese ripening processes take place predominantly on cheese proteins, this means that the relationship between the watery phase and the protein matrix phase is important in regulating enzymatic processes during ripening period (2, 3).

Cheese ripening caused by enzymatic coagulation of milk implies changes in proteins. This is the longest phase that can last up to 2 years, and during this period the texture and taste characteristic of the corresponding type of cheese develop (4).

Biochemical reactions that take place in cheese during ripening include glycolysis, lipolysis and proteolysis. In many varieties of cheeses, proteolysis is the most important and the most complex of all biochemical transformations that occur during ripening (4).

For the most types of cheeses and their variants, the initial hydrolysis of casein occurs by the action of the residual coagulant enzyme, and by the action of plasminin fewer extent. The products of these reactions are peptides of high molecular weight. Further degradation of these peptides takes place through coagulant enzymes, native enzymes, as well as enzymes of starter and non-starter cheese microflora. All this indicates that cheese ripening is essentially an enzymatic process (5, 6).

Traditional way of cheese production still occupies a very important place in the overall cheese production in the world and in our country. For the production of these types of cheeses, only animal origin rennet is used. It is mainly made from calves stomach, in which the most common enzymes are chymosin and pepsin, and their weight ratio depends on the animal age (5). Residual coagulant, which is, in a certain percentage retained in curd (0-15%), makes the main and essential contribution to cheese proteolysis during ripening, and thus to the development of taste and texture (7).

2. MATERIALS AND METHODS

The experiments in this paper were performed on Sjenica-Pester plateau, where Sjenica cheese is traditionally produced by autochthonous technology. The cheese is produced from fresh full-fat sheep's milk like the original Sjenicki and cow's milk in the Sjenicki type.

According to the plan of conducting the experiment in the wide area of Sjenica-Pester plateau, we selected 6 households that produce cheese with indigenous technology from sheep and cow milk in the following way:

The raw material for production is fresh, whole milk that is curdled with rennet immediately after milking at a temperature of 28-32 ℃, without adding any additives or starter cultures, for 50-60 minutes. Curd processing is simple. The curd is not cut, but transferred to cheesecloths where the whey is separated by squeezing for 4-8 hours by self-pressing or pressing under the pressure. Curd shaping is done in cheesecloths, after whey separation. The resulting cheese bundle is cut into slices, which are stacked and successively salted with dry coarse sea salt. Cheese ripening lasts for 30-40 days in salted whey, which is released by the cheese, or is added, thus providing anaerobic ripening conditions.

After the production of fresh cheese, a certain content of total proteins was determined, and we followed the changes in total proteins after 30, 60, 120, 180 days, which was the determined ripening period.

The analyzes were performed in the chemical laboratory of the Faculty of Agriculture in Belgrade, by the following method:

Percentage of total proteins by Kjeldahl method, IDF standard (8).

Procedure: About 0.5 g of cheese sample is put into a Clydal cuvette, then 10 ml of concentrated H_2SO_4 is added, and after that it is burnt on a hot plate (> 420 ℃) until the contents become clear. Then, the content is cooled to room temperature for about 25 min, and 75 ml of distilled water is added into it. Next, 25 ml of 4% boric acid is poured into the Erlenmeyer flask, and distillation is performed up to 150 ml of distillate into a Klejtek apparatus. The distillate is titrated with 0.1N H_2SO_4 until the green color disappears, ie. decolorization-a moment before the formation of pink color.

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The content of total nitrogen substances is calculated using the following formula:

$$UN = \frac{1{,}4007 \cdot (V1 - V2) \cdot c \cdot F}{m}$$

V1 - ml of H_2SO_4 spent for sample titration;

V2 - ml of H_2SO_4 spent for blank test;

C - normality of H_2SO_4 (0,1);

F - morality factor H_2SO_4;

m - mass of the measured cheese sample (g).

The total protein (TP) content is calculated when the total nitrogen (TN) is multiplied by a milk protein factor of 6.38%.

TP (%) = TN • 6.38

The extent of protein degradation by SDS-PAGE electrophoresis.

Statistical evaluation: The basic characteristics of a series of obtained data are shown through the mean values. The deviations of the individual data in each serie from the arithmetic mean of the corresponding serie, as well as the strength of their grouping around the mean, are shown through measures of variation (standard deviation and coefficient of variation). Student's t-test was applied to test the differences of the mean values (9). The SPSS statistics software (IBM, USA) was used.

3. RESULTS AND DISCUSSION

The most important and most complex changes during the ripening of cheeses occur to proteins. Therefore, ripening of cheese actually implies changes of proteins. The extent and dynamics of protein degradation are closely related to the technology of cheese production, ripening conditions, activity of coagulant enzymes, starters, non-starter microflora, etc.

If we talk about the dynamics of nitrogenous substances, and changes to proteins, it should be considered several factors in indigenous cheese production: milk is curdled fresh, without heat treatment, and it has a corresponding effect on the protein characteristics, as a changing substrate subject during ripening. Actually, to the cheeses, which are produced in traditional way, the basis of the protein matrix is casein, which easily subject to changes during ripening. Also, starter cultures are not added to these types of cheeses during the production, but the basic factors of ripening are enzymes of coagulants and non-starter-autochthonous microflora that naturally exist in milk.

The original raw material for Sjenica cheese is sheep milk. That raw material should be preserved as much as possible in the production of his type of cheese. Mirecki et al. (10) states that in the traditional production of Njegusi cheese, originality increasingly disappears, because sheep milk is increasingly replaced by cow, goat or a mixture of these types of milk.

Protein breakdown gradually takes place during ripening. In the first stage of protein breakdown, insoluble products are formed, while in the second stage, soluble but non-volatile products of separation are formed. They affect on consistency formation of white cheese. In the last, third phase of ripening, further decomposition of protein breakdown products results in the introduction of soluble and volatile products that affect the formation of the taste and smell of white cheese (11).

Table 1 and chart 1 shows the dynamics of total proteins during ripening of Sjenica cheese, and Table 2 and chart 2 shows the dynamics of total proteins in dry matter.

Based on the results from *Table 1*, it can be seen that the content of total proteins after production in fresh cheese was 13.43% for Sjenica, and 13.77% for the type of Sjenica cheese. The largest increase of total proteins in Sjenica cheese is recorded in the first 30 days of ripening (2.11%), when it reaches a maximum value of 15.54%, which is in the line with increase of cheese dry matter content. For the type of Sjenica cheese, an increase in total proteins of 2.78% is recorded during 60 days of ripening, when it reaches a maximum of 16.55%. After 30 days, for Sjenica cheese, or 60 days for the type of Sjenica cheese, a slight continuous decrease of total proteins was observed in both types of cheeses. After 180 days of ripening the content of total proteins was 12.78% for Sjenica cheese and for the type of Sjenica it was 13, 92%. The decrease happened due to transfer of a part of soluble nitrogenous substances from cheese to ripening brine.

The obtained results are in accordance to the results from this cheese group, stated by the following authors: white cheese 15.12%, Živić et al. (12);

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Homolje cheese, 17.37%, Maćej et al. (13); Sjenica, 17.34%, Savić (14); Polimlje-Sjenica cheese, 14.04%, Dozet et al. (15); Homolje cheese 17.32%, Zlatar cheese 16.91%, Jovanović et al. (11); Svrljig cheese 16.32%, Maćej et al. (13); Teleme, 16.42%, Abdelsalam et al. (16);

How important is a regular ripening process on the proteolysis course, Bontinis et al. (17) state that the course of proteolysis determines the development of texture and cheese sensory properties.

Activity of chimosin during ripening and the amount of residual chymosin are influenced by several factors: the amount of added rennet, the water content in the cheese and the salt content in the cheese. The pH value during curd processing has a great importance in the activity and amount of retained rennet. The lower the pH value of curd, the higher the percentage of coagulant retained in cheese, and therefore its activity during cheese ripening is higher (18). This statement is consistent with the pH conditions characterized by Sjenica cheese.

Sensory properties are also affected by free lipid acids. Barac et al. (19) analyzing the lipid acid content of three types of white cheeses in brine from the Republic of Serbia (Homolje cheese, Svrljig cheese, Zlatar cheese) has found that the content of saturated lipid acids was the lowest in Sjenica cow cheese, and the content of desired lipid acids (46-34%) was the best. Therefore, this type of cheese had the most favorable health aspects.

Protein degradation products contain significant antioxidants, ACE inhibitors, and antimicrobial properties and potential. The main ingredients responsible for the antioxidant properties of mature white cheeses in brine, are of low molecular weight peptides and amino acids. Considering the fact that they have other properties, traditional white cheeses play a significant role in maintaining the human antioxidant and antihypertensive immune system (20).

Considering that the autochthonous technology of Sjenica cheese is characterized by variability in the method of production and quality, significant variations of the results in the data series are observed, which is reflected in the values of minimum and maximum. When such variable results in series are reduced to an average value, the differences between them are minimal. Therefore, differences were not statistically significant among the cheeses for all ripening periods.

Electrophoretyc testing of protein change during cheese ripening

The proteolytic processes that take place during the ripening of indigenous white cheeses in brine are very complex, which is a consequence of some specificities in the production of these cheeses and in a large extent are dependent on the indigenous present microflora. Indigenous white cheeses in brine are made from raw whole fat milk without heat treatment and additives. This means that native protein ases of milk have a significant role in proteolytic processes during ripening. In addition, starter cultures are not used in the production of these cheeses, and besides the coagulants, enzymes of native microflora play a significant role. Among other factors, these factors are essentially the basis of differences in the chemical composition of indigenous white cheeses in brine. The large differences in composition are the consequence of the action of different indigenous microflora (21).

The SDS-polyacrylamide gel electrophoresis was performed as the aim of the experiment, in order to obtain a more complete picture of ripening of Sjenica cheese and the type of Sjenica cheese. The picture of the electrophoregram shows that the proteolytic changes mostly occurred during the first 60 days of ripening.

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Table 1. Dynamics of total proteins during cheese ripening (%)

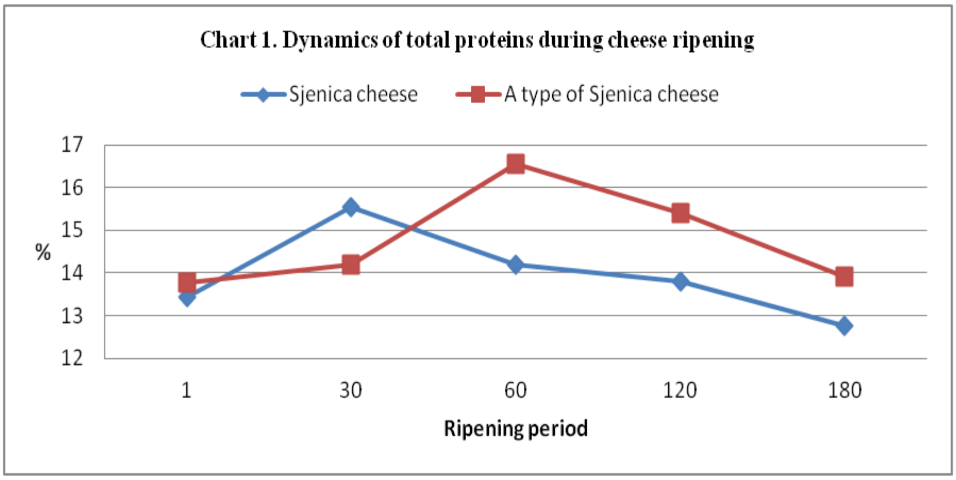
Type of cheese	Parameters	Ripening period (days)				
		1	30	60	120	180
Sjenica cheese	*min*	11.33	14.08	11.46	10.94	10.88
	max	15.10	17.28	17.28	15.55	15.81
	$\bar{x}$ *(n=6)*	13.43	15.54	14.21	13.79	12.78
	Sd	1.57	1.30	2.29	1.93	1.84
	CV(%)	11.66	8.38	16.09	13.96	14.40
The type of Sjenica cheese	*min*	13.18	14.21	15.23	13.95	12.35
	max	14.53	14.21	17.79	19.01	17.47
	$\bar{x}$ *(n=6)*	13.77	14.21	16.55	15.39	13.92
	Sd	0.51	0.00	0.85	1.88	2.03
	CV(%)	3.69	0.00	5.12	12.18	14.61

Theoretical values of the t-arrangement refer to the number of degrees of freedom (df) = 10 and are p < 0.05 = 2.28; p < 0.01 = 3. 16; t-calculated by days: 1 = 0. 58; 30 = 2. 08; 60 = 3. 15; 120 = 2. 02: 180 = 1. 44.

Table 2. Dynamics of total proteins in dry matter.

Type of cheese	Parameters	Ripening period (days)				
		1	30	60	120	180
Sjenički cheese	*min*	30.13	29.44	25.63	24.18	25.30
	max	38.46	35.03	34.00	29.66	30.67
	$\bar{x}$ *(n=6)*	34.18	32.18	28.72	27.12	28.09
	Sd	3.20	1.94	2.88	2.38	2.35
	CV(%)	9.36	6.01	10.04	8.77	8.35
The type of Sjenički cheese	*min*	29.02	28.35	31.11	26.43	26.46
	max	35.46	31.97	36.48	34.48	37.59
	$\bar{x}$ *(n=6)*	32.05	30.19	34.22	30.61	29.47
	Sd	2.21	1.62	1.92	2.67	4.13
	CV(%)	6.89	5.35	5.62	8.72	14.02

Theoretical values of the t-arrangement refer to the number of degrees of freedom (df) = 10 and are p < 0.05 = 2.22; p < 0.01 = 3. 16< t-calculated by days: 1 = 2.26*; 30 = 2.61*; 60 = 6.17**; 120 = 3.83**: 180 = 1.33.



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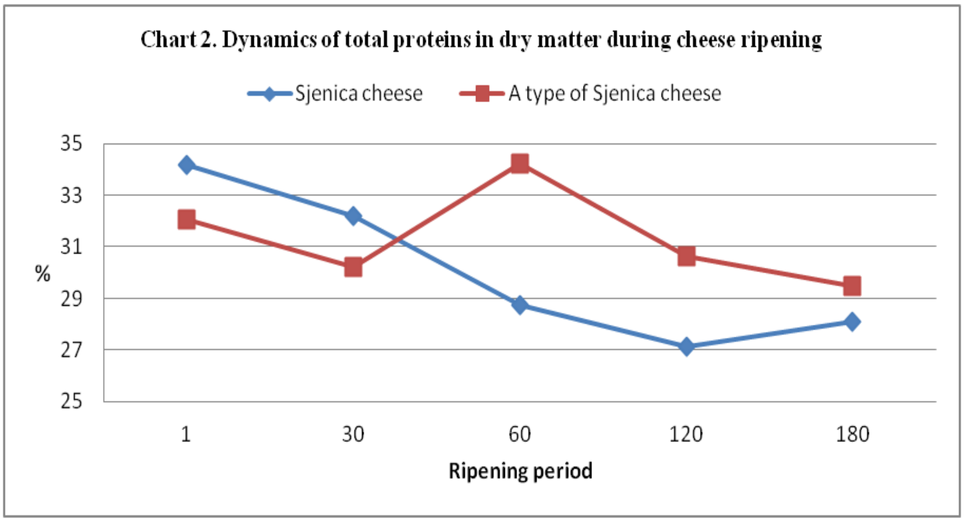

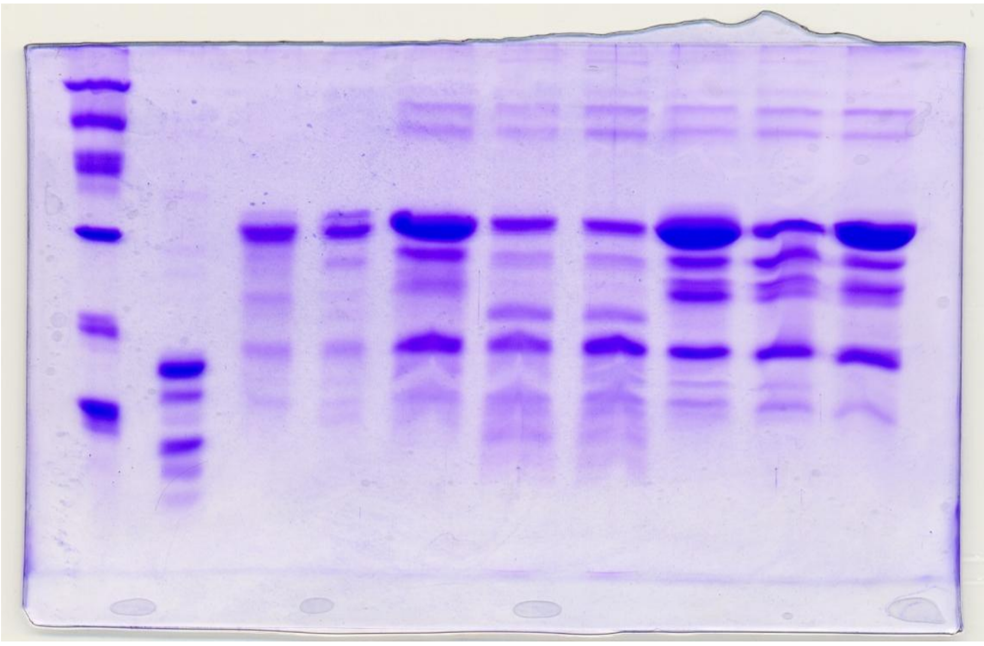

Figure 1. Electrophoregram 1 sheep cheese sample and 2 cow cheese samples (k1 and k2) after 1 day, with casein and proteins of known molecular weight (from left to right: O_1, O_2, O_3, O_4, O_5, O_6 – sheep's cheese samples 1 day old, K_1, K_2, - cow's cheese samples 60 days old, 9-β- kazein, 10- low molecular weight standard)

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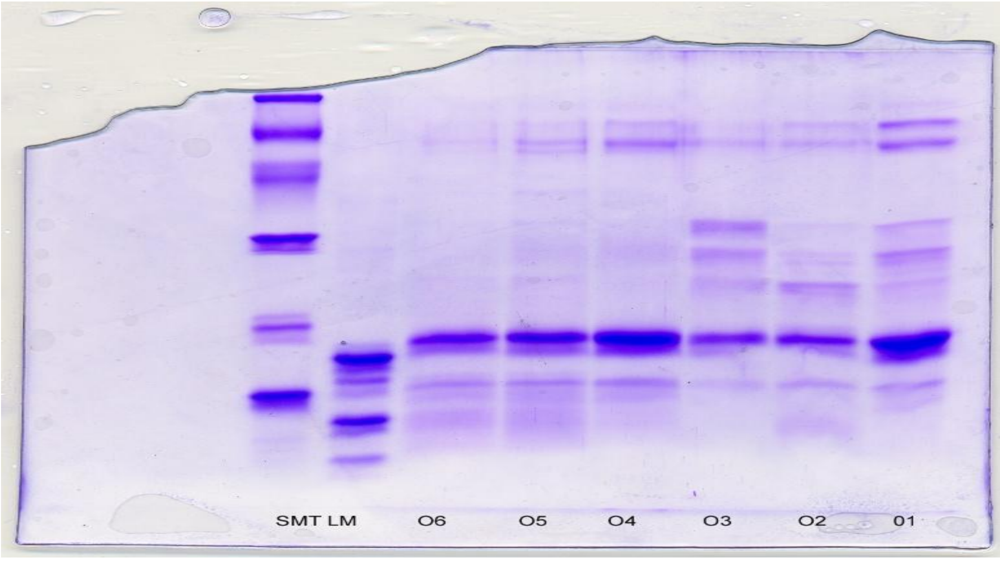

Figure 2. Electrophoregram 2 sheep cheese samples after 60 days of ripening, with casein and proteins of known molecular weight (O_1, O_2, O_3, O_4, O_5, O_6– samples of sheep's cheese 60 days old, LM-β- kazein, SMT-low molecular weight standards)

4. CONCLUSIONS

Proteins are the most important parameter of milk, which affects the course of coagulation. The composition and technological properties of curd form structure basis and rheological characteristics of cheese. Sjenica cheese, which is produced in the traditional way by enzymatic coagulation using rennet, without addition of pure cultures, indicates that the key role during ripening is played by rennet enzymes and non-starter microflora that is naturally present in milk. Cheese ripening is a typical enzymatic process, and the most important and complex changes occur on proteins. Changes to proteins during ripening determine the texture and sensory properties of mature cheese. The biggest changes in total proteins occurred in Sjenica cheese during the first 30 days of ripening, and in the type of Sjenica cheese in the first 60 days of ripening. After this period, there is a slight continuous decrease of total proteins in both types of cheeses, as a result of nitrogenous substances transition from cheese to brine. Electrophoretic tests showed that the proteins were mostly degraded during the first 60 days of ripening, after which the fractions appear as diffuse zones in traces.

REFERENCES

1. *Guinee T.P., Mulholland E.O., Jelly J., Callaghan D.J.O. Effect of protein-to-fat ratio of milk on the composition, manufacturing efficiency, and yield of Cheddar cheese. J Dairy Sci. 2007, 90, 110-123.*
2. *Fox P.F. and Cogan T.M. Cheese: scientific highlights of the 20th century. In 6th Cheese symposium. Ed. by Cogan, TM, McSweeney, PLH and Guinee, TP, Moorepark, pp. 83-121, 2000.*
3. *Walstra P., Noomen A., Geurts T.J. Dutch-Type varieties in cheese: Chemmistry, physics and microbiology. Volume 2. Major cheese groups. Second edition. Chapter 2, 39-82. Ed. Fox, P. F. Chapman & Hall, London and New York, 1993.*
4. *Upadhyay V.K., McSweeney P.L.H., Magboul A.A.A., Fox P.F. Proteolysis in Cheese During Ripening, Pages 390-434: Chemistry Physics and Microbiology, Third edition, Vol. 1. P.F. Fox, P.L., H. McSweeney, T.M. Cogan, and G.T.P., ed. Elsevier Applied Science, London, 2004.*
5. *Sousa M.J., Ardo Y. and McSweeney, P.L.H. Advances in the study of proteolyses during cheese ripening. International Dairy Journal 2001,11: 327-345.*
6. *Beresford T.P., O Reilly C., O Connor P., Murphy P.M. and Kelly A. VTT Symposium "Fresh novel foods by high pressure". Ed. By Aution, K., Helsinki, 103-114, 1998.*

ISBN 978-86-7520-611-8

7. Awad S., Luthi-Peng Q.-Q. and Puhan Z. Proteolytic Activities of Chymosin and Porcine Pepsin on Buffalo, Cow and Goat Whole and β-Casein Fractions. Journal of Agricultural and Food Chemistry 1998, 46 (12): 4997-5007.
8. Miočinović J. Milk Technology-Practicum, University of Belgrade, Faculty of Agriculture. Belgrade, 2013.
9. Stanković J., Ralević N., Ljubanović-Ralević I. Usage of statistics in agriculture, modern administration, Belgrade, 1989.
10. Mirecki S., Popović N., Antunac N., Mikulec N., Plavljanić D. Technology production and some quality parameters of Njeguši cheese, Mljekarstvo 2015, 65 (4) 280-286.
11. Jovanović S., Maćej O., Barać M. Characteristics of autochthonous production of Sjenica cheese at Sjenica-Pester plateau region Biotechnology in Animal husbandry 2004, 20 (1-2). p 131-139.
12. Živić N. Energy and quality value of milk and dairy products from Nis region, doctoral dissertation, Faculty of Agriculture, Sarajevo, 1989.
13. Maćej O., Dozet N., Jovanović S. Characteristics of indigenous production of Sjenica-Homolj, Zlatar and Svrljig white cheeses, Monograph "Autochthonous white cheeses in brine", Ur. Maćej, O. and Dozet, N. 33-65. Faculty of Agriculture, Belgrade, 2006.
14. Savić Ž. Comparative research of the quality of Sjenica cheese produced by autochthonous technology and in industrial conditions, Master's thesis, Faculty of Agriculture, Belgrade, 1997.
15. Dozet N., Adžić N., Stanišić M., Živić N. Indigenous dairy products. Agricultural Institute, Podgorica, Silmir, Belgrade, 1996.
16. Abd El Salam M.H., Alichandis E., Zerfiridis G.K. Domiati and Feta Tupe Cheeses, P. F. Fox (Editor): Cheese, chemistry, physiens and microbiology, London, 1993.
17. Bontinis T.G., Mallatou H., Pappa E.C, Massouras T., Alichanidis E. Study of proteolysis, lipolysis and volatile profile of a traditional Greek goat cheese (Xinotyri) during ripening. Small Ruminant Research 2012, 105 (1-3): 193-201.
18. Sheeha. J.J., Oliveira J.C., Kelly A.I., Kelly A.L., McSweeney P.L.H. Effect of cooking temperature on primary proteolysis and predicted residual chymosin activity of a semi-hard cheese manufactured using thermophisms cultures. International Dairy Journal 2007, 17 (7): 826-834.
19. Barać M., Kresojević M., Špirović-Trifunović B., Pešić M., Vučić T., Kostić A., Despotović S. Fatly acid profiles and mineral content of Serbian traditional white cheeses in brine, Dairy 2018, 68 (1) 37-45.
20. Barac M., Smiljanic M., Zilic S., Pesic M., Stanojevic S., Vasic M., Vucic T., Kostic A. Protein profiles and total antioxidant capacity of water soluble and insoluble protein fractions of white cow cheese at different stage of ripening. Dairy 2016, 66, 187-197.
21. Barać M., Jovanović S., Maćej O. Proteolytic changes during ripening of "Indigenous white cheeses in brine", Faculty of Agriculture, Belgrade, 2006.

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Article
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TECHNOPATIES IN DAIRY COWS - PERITARSAL LESIONS

TEHNOPATIJE KOD MLEČNIH KRAVA – PERITARZALNE LEZIJE

David Slatinek[1], Andrej Toplak[2], Maja Prevolnik Povše[1], Marko Cincović[3], Rok Marzel[4], Jože Starič[4]

[1] Faculty of Agriculture and Life Sciences, University of Maribor, Maribor, Slovenia
[2] Agricultural advisory service Ptuj, Chamber of Agriculture and Forestry of Slovenia, Ptuj, Slovenia
[3] Faculty of Agriculture – Department for veterinary medicine, University of Novi Sad, Novi Sad, Serbia
[4] Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia
Correspondence: joze.staric@vf.uni-lj.si

M.P.P. https://orcid.org/0000-0002-7235-6810, M.C. https://orcid.org/0000-0002-5683-8983, R.M. https://orcid.org/0009-0000-2753-3374, J.S. https://orcid.org/0000-0002-5156-5277

ABSTRACT

Technopathies in dairy cows represent a complex interplay between technological advancements, management practices, and animal health. While technological innovations have undoubtedly improved efficiency and productivity, they also introduce new challenges, particularly in relation to the health and welfare of dairy cows. Lameness associated with claw pathology is one of a prevalent technopathy in dairy cows. However, it can be associated also by other pathologies, including peritarsal lesions. This paper aims to explore the peritarsal lesions in dairy cows, their prevalence in selected Slovenian herds and their connection to flooring and cubicle type. Tarsal region was evaluated in 16 dairy herds in NE Slovenia. We assessed 881 dairy cows. Type (alopecia, erosions, decubitus, swelling) and size (up to 3 cm^2, < 20 cm^2, < 50 cm^2 and >50 cm^2) of lesions were documented. In all herds type of flooring and cubicles were recorded including their size and occupancy %. Results were statistically evaluated using descriptive statistics, correlations and ANOVA analysis. Significance was set at $p<0.05$. Out of 881, 373 (42.3%) had peritarsal lesions. The prevalence on herd level was from slightly above 2 to more than 70%. We noticed that peritarsal lesions are more prevalent in herds with high cubicles, than in those with deep cubicles. Cubicle occupancy rate statistically non-significantly influenced peritarsal health. Results of the study surprised us, as we have expected far lower prevalence of peritarsal lesions. A positive fact is that we also had a herd with very favorable status concerning hock lesions, which proves that much better situation in this issue can be achieved. Collaborative efforts among researchers, veterinarians, and farmers are essential for identifying key issues leading to technopathies, implementing effective mitigation strategies, and promoting sustainable dairy farming practices.

Keywords: peritarsitis, cattle, trauma, technology induced pathology

SAŽETAK

Tehnopatije kod mlečnih krava predstavljaju složenu interakciju između tehnološkog napretka, prakse upravljanja i zdravlja životinja. Dok su tehnološke inovacije nesumnjivo poboljšale efikasnost i produktivnost, one takođe uvode nove izazove, posebno u vezi sa zdravljem i dobrobiti muznih krava. Hromost povezana sa patologijom papaka je jedna od rasprostranjenih tehnopatija kod mlečnih krava. Međutim, može se povezati i sa drugim patologijama, uključujući peritarzalne lezije. Ovaj rad ima za cilj da istraži peritarzalne lezije kod muznih krava, njihovu prevalenciju u odabranim slovenačkim stadima i njihovu povezanost sa podom i tipom boksa. Tarsal region je evaluiran u 16 muznih stada u SI Slovenije. Ocenili smo 881 kravu muzaru. Dokumentovani su tip (alopecija, erozije, dekubitus, otok) i veličina (do 3 cm^2, < 20 cm^2, < 50 cm^2 i >50 cm^2) lezija. U svim stadima evidentirani su tipovi podova i objekata

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uključujući njihovu veličinu i popunjenost u %. Rezultati su statistički procenjeni korišćenjem deskriptivne statistike, korelacija i ANOVA analize. Značajnost je postavljena na p<0,05. Od 881, 373 (42,3%) imalo je peritarzalne lezije. Prevalencija na nivou stada bila je od nešto iznad 2 do više od 70%. Primetili smo da su peritarzalne lezije zastupljenije u stadima sa visokim boksovima, nego u onima sa dubokim boksovima. Stopa zauzetosti objekta statistički nije značajno uticala na zdravlje peritarza. Rezultati studije su nas iznenadili, jer smo očekivali daleko manju prevalenciju peritarzalnih lezija. Pozitivna činjenica je da smo imali i stado sa veoma povoljnim statusom u pogledu lezija skočnog zgloba, što dokazuje da se po ovom pitanju može postići mnogo bolja situacija. Zajednički napori između istraživača, veterinara i farmera su od suštinskog značaja za identifikaciju ključnih pitanja koja dovode do tehnopatija, implementaciju efikasnih strategija za ublažavanje uticaja i promovisanje održivih praksi uzgoja mleka.

Ključne reči: peritarzitis, goveda, trauma, tehnologijom izazvano oboljenje

INTRODUCTION

Technopathies, or technology-induced pathologies, are increasingly becoming a concern in dairy farming as an advance in the sector reshape the industry. Lameness associated with claw pathology is one of a prevalent technopathy in dairy cows, often exacerbated for example by the introduction of concrete flooring in housing systems and the prolonged periods of standing associated with automated milking systems. However, there are also other causes of lameness, such as hock lesions associated with trauma from laying surfaces. Lameness not only affects the welfare of cows but also leads to decreased milk production and reproductive performance. Strategies such as providing comfortable resting areas, regular hoof trimming, and optimizing flooring surfaces can mitigate the risk of lameness. In European Union (EU) countries, including Slovenia, consumers not only want a wider choice of animal-friendly food products, but also more information about how farm animals are reared and stricter animal welfare than is currently practiced. It is therefore to be expected that more animal products will be labeled for animal welfare in Slovenia in the future, as is already the case in some EU countries. In this context, the prevention of technopathies will come to the fore in the near future, as the regulations are going to require action when they will become noticed.

It is important that veterinary and animal science profession is well prepared for these changes as they are going to be vital in advising farmers when they seek solutions to improv situation concerning technopathies. All this resulted in decision to study prevalence of technopathies in selected dairy farms and connect them to farm characteristics. In this work peritarsal lesions are going to be studied.

MATERIALS AND METHODS

Tarsal region of cows was evaluated in 16 dairy herds with free stall type housing system in North-East Slovenia. In total 881 dairy cows were assessed. Type (alopecia, erosions, decubitus, swelling) and size (up to 3 cm2, < 20 cm2, < 50 cm2 and >50 cm2) of lesions were documented. In all herds cubicles were recorded including their size and occupancy index (number of cubicles/number of cows).

RESULTS

Peritarsal lesions were noticed in 373 cows (42.3%) out of 881 checked. The prevalence on herd level was from slightly above 2 to more than 70%. We noticed that peritarsal lesions are more prevalent in herds with high cubicles (more than 60% animals were affected), than in those with deep cubicles (less than 30% animals were affected). Interestingly higher occupy rate (<1) was associated with less peritarsal lesions though there was no statistically significant difference.

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Figure 1. Extensive swelling, alopecia and erosions of peritarsal region in dairy cow

Results were statistically evaluated using descriptive statistics, correlations and Anova. Significance was set at $p<0.05$.

DISCUSSION

Authors encountered difficulties in measuring the occurrence and severity of peritarsal injuries, as it is very hard to say what the extent and type of injury means for the animal. This is probably also a reason that there are not many scientific works about this topic available in the literature and that its use in animal welfare assessing is limited. Results of the study surprised us, as we have expected far lower prevalence of peritarsal lesions. A positive fact is that we also had a herd with very favorable status concerning hock lesions, which proves that much better situation in this issue can be achieved. Further much of the variation is also a result of the fact that a lesion can range from severe and extensive to a minor skin abrasion. A Canadian study found even that more than 75% of dairy cows had some form of hock lesion and they considered all forms, from minor to major (Weary & Taszkun, 2000). Judgement of lesions is not straight forward and further research evaluating the gravity of peritarsal lesions for animal welfare using additional more precise equipment is needed.

High cubicles caused more peritarsal lesions than deep cubicles. When they compared cows in large, well-bedded stalls with cows in narrow tie stalls with concrete surfaces, they found that the cows in the large, well-bedded stalls spent approximately four hours more per day lying down (Haley et al., 2001). This clearly indicates that the lying area was more comfortable. Thus, the main difference between cubicle types is just the presence or absence of bedding (deep cubicles have much more bedding than high cubicles, which had only rubbber mats and minimal or no bedding material. Our opinion is that the type of lying surface and the amount of bedding material play a pivotal role in the prevalence of peritarsal lesions and not the type of cubicles.

The result that there are fewer peritarsal lesions in crowded herds is controversial. We assume that this is due to other factors, such as a better type of cubicles, younger cows in a herd, etc. Our results show that statistical indicators need to be evaluated very carefully and that all possible influences need to be considered before drawing conclusions.

According to literature lameness associated with upper-leg problems seems to have a larger effect on

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the cows' feed intake and milk production than does lameness associated with hoof problems (Bareille et al., 2003). However, this cannot be proven with our study, but must be considered in future research, as this information has a decisive influence on the fate of cows in a herd, their longevity.

While technology offers promising solutions for improving efficiency and productivity in dairy farming, where we notice continuous improvements. However longevity of animals is lacking behind, so it is imperative to prioritize the welfare of cows and minimize the negative impacts of technological interventions. Collaborative efforts among researchers, veterinarians, and farmers are essential for identifying key issues leading to technopathies, implementing effective mitigation strategies, and promoting sustainable dairy farming practices. Additionally diagnostic methods for evaluation of peritarsal lesions need improvement.

LITERATURE

1. *Bareille N., Beaudeau F., Billon S., Robert A., Faverdin P. Effects of health disorders on feed intake and milk production in dairy cows. Livestock Production Science 2003, 83(1), 53–62. https://doi.org/10.1016/S0301-6226(03)00040-X*
2. *Haley D.B., De Passillé A.M., Rushen J. Assessing cow comfort: effects of two floor types and two tie stall designs on the behaviour of lactating dairy cows. Applied Animal Behaviour Science 2001, 71(2), 105–117. https://doi.org/10.1016/S0168-1591(00)00175-1*
3. *Weary D.M., Taszkun I. Hock Lesions and Free-Stall Design. Journal of Dairy Science 2000, 83(4), 697–702. https://doi.org/10.3168/JDS.S0022-0302(00)74931-9*

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UPOTREBA ULTRAZVUČNE DIJAGNOSTIKE I INFRACRVENE TERMOGRAFIJE U DIJAGNOSTICI OBOLJENJA EKSTREMITETA KOD GOVEDA

THE USE OF ULTRASOUND DIAGNOSTICS AND INFRARED THERMOGRAPHY IN DIAGNOSIS OF LIMB DISEASES IN CATTLE

Jovan Spasojević[*a], Marko Cincović[a], Mira Majkić[a], Jovan Stanojević[a], Miloš Gurdeljević[b], Vladimir Gurjanov[b]

[a] Department of Veterinary medicine, Faculty of Agriculture, University of Novi Sad, Novi Sad, Trg Dositeja Obradovica 8, Republic of Serbia
[b] DOO Pik Bečej Veterina, Bečej, Novosadska ulica 155, Republic of Serbia
[*]Corresponding author: jovan.spasojevic1984@yahoo.com; spasojevic.jovan@polj.uns.ac.rs

J.S. https://orcid.org/0000-0001-5654-5796, M.C. https://orcid.org/0000-0002-5683-8983, M.M. https://orcid.org/0009-0007-0110-7327, J.St. https://orcid.org/0000-0001-7611-6876

SAŽETAK

Oboljenja lokomotornog sistema goveda kao što su: artritisi, tendinitisi, tenovaginitisi, burzitisi/higromi i apscesi, imaju značajnu ulogu u zdravstvenom i proizvodnom statusu životinja, jer pored toga što direktno narušavaju zdravlje životinja, uzročno-posledično dovode i do pada proizvodnje mleka, zasušivanja životinja usled potrebe lečenja, te na kraju i do trajnog isključivanja grla iz proizvodnog procesa i ekonomskog iskorišćavanja životinja. U skladu sa prethodno navedenim, može se lako zaključiti da pravovremena dijagnostika predstavlja jedan od značajnih preduslova za adekvatno lečenje obolelih životinja, a samim tim i njihovo zadržavanje u proizvodnom procesu. Cilj ovog istraživanja bio je da se ustanove prednosti i mane ultrazvučne dijagnostike i infracrvene termografije u dijagnostici oboljenja ekstremiteta kod goveda, u terenskim uslovima rada. Istraživanje je sprovedeno na komercijalnim mlekarskim farmama, na grlima sa prethodno klinički dijagnostikovanim nekim od sledećih oboljenja lokomotornog sistema: artritisi, apscesi i higromi/burzitisi. U skladu sa postavljenim ciljevima istraživanja, mogu se izdvojiti dva najvažnija rezultata ovog istraživanja: 1. Izvođenje infracrvene termografije predstavlja jednostavniju i bržu dijagnostičku proceduru, bez direktnog kontakta sa pregledanom životinjom, što ujedno smanjuje i nivo stresa kod životinje. Međutim, termografijom se mogu otkriti anatomske, topografske i funkcionalne promene, ali ne i tačna lokalizacija ni bliža karakterizacija samog procesa; 2. Prilikom izvođenja ultrazvučnog pregleda u evaluaciji eksudativnih oboljenja lokomotornog sistema goveda, uzimajući u obzir određene kriterijume ultrazvučnog pregleda (anatomska lokacija lezije, ehogenost prisutnih struktura, veličina lezije, osobenost ivica lezije i šupljine ispunjene eksudatom, prisustvo fenomena pretakanja i akustičnih artefakta), moguće je dobiti određene podatke o količini, vrsti i sadržaju prisutnog eksudata, te na taj način bliže okarakterisati prirodu patološkog procesa, kao i mogućnosti lečenja.

Ključne reči: krava, oboljenja ekstremiteta, ultrazvučna dijagnostika, infracrvena termografija

ABSTRACT

Diseases of the locomotor system of cattle, such as: arthritis, tendinitis, tenovaginitis, bursitis/hygromas and abscesses, have a significant role in the health and production status of animals, because in addition to directly impairing the health of animals, they cause and also lead to drop in milk production, drying off the animals due to the need for treatment, and to the permanent exclusion from the production process and economic exploitation of animals. According to all above, it could be easily concluded that right time diagnosis is one of the most important prerequisites for the adequate treatment of sick animals, and therefore their retention in the production process. The aim of this study was to establish the advantages and disadvantages of ultrasound diagnostics and infrared thermography in the diagnosis of some locomotor diseases in cattle, in field working conditions. This study was conducted on commercial dairy farms, on cows previously clinically diagnosed with some of the following diseases of the locomotor system: arthritis, abscesses and hygromas/bursitis. According to the aim of this study, two most important results could be singled out: 1. Performing infrared thermography represents a more simple and faster diagnostic procedure, without direct contact with the examined animal, which also reduces the level of stress in the animal. However, thermography can detect anatomical, topographic and functional changes, but not the exact localization or a closer characterization of the process itself; 2. When performing an ultrasound examination in the evaluation of exudative diseases of the locomotor system of cattle, taking into account certain criteria of the ultrasound examination (anatomical location of the lesion, echogenicity of present structures, the size of the lesion, the characteristics of the edges of the lesion and the cavity filled with exudate, the presence of overflow phenomena and acoustic artifacts), it is possible to obtain certain data on the amount, type and content of the present exudate, and in this way more closely characterize the nature of the pathological process, as well as the possibilities of treatment.

Key words: cow, locomotor diseases, ultrasonography, infrared thermography

INTRODUCTION

Some diseases and disorders of locomotor system in cattle such as: bursitis, hygromas, abscesses and arthritis, are characterized by extensive soft tissue swelling and presence of inflammatory exudation (1,2). Those diseases, with the nature of their pathoanatomical and pathophysiological processes, could lead to drop in milk production, drying off the animals due to the need for treatment, to the permanent exclusion from the production process and economic exploitation of animals (3). According to all mentioned above, it could be easily concluded that right time diagnosis is one of the most important conditions for the adequate treatment of sick animals, and therefore their retention in the production process.

Ultrasonography is one of the most useful diagnostic tools for evaluation of diseases of locomotor system in cattle which are characterized by soft tissue swelling and accumulation of exudate. Ultrasound allows the evaluation of echogenicity of the content, extent of effusion, type of the border of the lesion, cavity and swelling, presence of flow phenomena and presence of ultrasound artefacts (1,2,4,5). According to these criteria, ultrasonography provides certain information about the nature of the content in the affected area, especially for fluid and semisolid masses, but it cannot definitively characterize the composition of the content and the type of effusion by itself (1,2). Definitive answer about the type of effusion and its characteristics is provided by obtaining samples by centesis and laboratory analysis of aspirated fluid (1,2,6-9).

Infrared thermography (IRT) finds its application primarily in the diagnosis of diseases of the locomotor system (primarily diseases of the distal parts of the extremities - arthritis and diseases of the paws), then in the diagnosis of diseases of the udder and skin (10,11,12,13). With development of inflammatory processes, the body's defense mechanisms are activated, and those processes are manifested by the movement of heat through the tissue, greater blood flow and increased temperature of the surface (11,12,13). These changes in temperature of the body surface (skin surface) are detected by IRT camera, and according to their different presentations, those changes could indicate different stages of inflammatory processes.

The aim of this study was to establish the advantages and disadvantages of ultrasound diagnostics and infrared thermography in the diagnosis of some locomotor diseases in cattle, in field working conditions.

MATERIALS AND METHODS

During our mobile clinic work, in the period between 2014 and 2016, 50 cows with superficial swellings on their limbs were observed by ultrasound examination. After ultrasound examination was done, samples of effusions were collected by centesis and sent for laboratory analysis of aspirated fluid. Clinical and orthopaedic examinations were performed to identify “the region of interest” for ultrasound examination. It was found that 4 of 50 cows had swellings on their hind limbs at the femoral region, and 46 of 50 cows had swellings at the carpal or tarsal region. Before ultrasound examination was conducted, the skin over the affected area was clipped and shaved. Ultrasound examination was conducted with an ultrasound machine (*Esaote Pie Medical®, Netherlands*) using an 8 MHz linear transducer, in real-time. Presence of flow phenomena and echogenicity of the content were evaluated. Four ultrasound images were taken for every case of swelling and analyzed by using image analyzer software ImageJ (*National Institutes of Health, Bethesda, Maryland, USA*). Values of pixel distribution within the grayscale (0 = black, 255 = white), which present values of mean echogenicity (ME), were determined for every ultrasound image in that part which present a liquid content of effusion. These values were quantified by randomly selecting 10 circles with a size of 15 pixels on every ultrasound image (*Fig. 1*). Average values of ME were determined for every case of swelling. Fluid sampling was performed after clinical and ultrasound examination. Before centesis, the skin was disinfected with 10% povidone-iodine solution. The fluids were obtained using a syringe and EDTA vacutainers, stored in a portable fridge and analyzed in the laboratory within 4 h. Next laboratory findings were measured and analyzed: Concentration of total proteins (Tp), concentration of glucose (Glc), concentration of uric acid, number of leukocytes (Le), percentage of neutrophils (Ne), number of erythrocytes (Er), specific weight, viscosity, colour, clarity and Gram stain. Concentration of Tp, Glc and uric acid were measured using Analyzer A15 (*Biosystems S.A., Barcelona, Spain*). Number of Le and Er were analyzed using ADVIA 120 haematology system (*Siemens, Germany*).

Descriptive statistic values based on average ME values and distribution of frequency for average ME values were calculated. The correlation between average ME values and descriptive characteristics of the effusions such as viscosity (very viscous, viscous, sero-viscous, serous), colour (milky yellow, yellow, red), clarity (blurry, blurred, slightly cloudy, clear) and Gram stain (presence of bacteria, absence of bacteria), was tested using $\chi 2$ test and $2 \times k$ contingency tables, so the significance of difference in the proportion of cows that have certain characteristics of effusions in the groups based on average ME values above and under the median value determined by descriptive statistics has been investigated. All statistical analyses were performed with Statistica (*TIBCO Software Inc., USA*) and Microsoft Office Excel 2007 (*Microsoft Corporation, USA*).

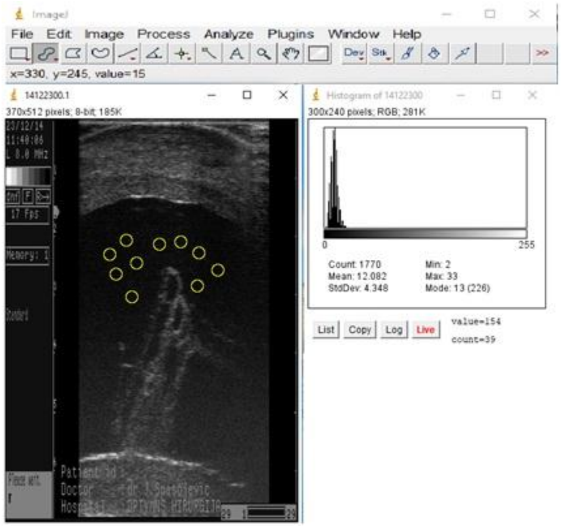

Figure 1. Determination of mean echogenicity (ME)

During our mobile clinic work, in the period July – November 2023., three cows with suspected arthritis and significant clinical findings of pain and swellings were examined by infrared thermography (IRT). IRT was conducted by infrared camera Testo 865 (*Testo, Bangkok, Thailand*). Suspected areas were recorded in 1-1,5 m distance between clinician and animals.

RESULTS

According to the aim of this paperwork, here are presented results of descriptive statistic values based on average ME values and distribution of frequency for average ME (*Table 1* and *Fig. 2*).

For the purpose of field condition work, in this paperwork are presented results of correlation between average ME values and descriptive characteristics of the effusions such as viscosity (very viscous, viscous, sero-viscous, serous), colour (milky yellow, yellow, red), clarity (blurry, blurred, slightly cloudy, clear) and Gram stain (presence of bacteria, absence of bacteria) (*Table 2*). The results in *Table 2*. showed that there is a significant difference in the proportion of cows with certain characteristics of effusions in function of that whether the average ME values were high or low. In cows, in which higher average ME values were found, very viscous and viscous consistency, milky yellow color, and poor transparency (blurry) of the effusions dominated and in a large proportion of the samples there was presence of bacteria. On the other hand, in cows with lower average ME values of ultrasound findings, serous, yellow color, and cloudy or clear effusions dominated, and also absence of bacteria in a large portion of the samples.

In *Table 3. And Fig. 3-5* are presented results of clinical and IRT results of three examined cows.

Table 1. Descriptive statistic values based on average ME values

Variable	Descriptive Statistics									
	N	Mean	Median	Min	Max	Lower Quartil	Upper Quartil	Std. Dev.	Skewness	Kurtosis
Mean Echogenocity (ME)	50	13,02	9	5,6	34,62	7	14	8,95	1,51	0,86

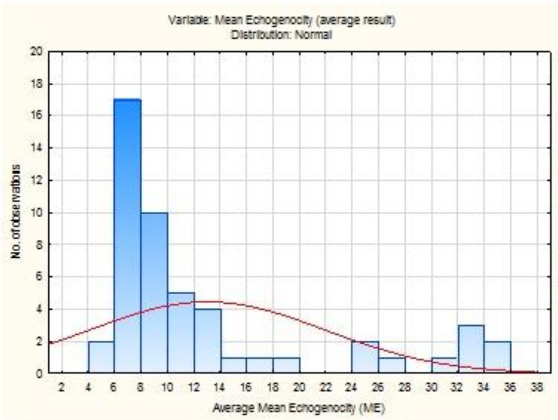

Figure 2. Distribution of frequency for average ME values

Table 2. Connection between ME value and physical characteristic and presence of bacterial cell in exudate

Exudate characteristics	Category	Number of cows according to average ME values		χ^2 test
		Above the median value	Under the median value	
Viscosity	very viscous	7	0	P<0.0005
	viscous	7	1	
	sero-viscous	6	7	
	serous	5	17	
Colour	milky yellow	9	0	P<0.005
	yellow	10	18	
	red	6	7	
Clarity	blurry	16	4	P<0.005
	blurred	7	10	
	slightly cloudy	2	3	
	clear	0	8	
Presence of bacteria (Gram stain)	Presence of bacteria	16	3	P<0.0005
	Absence of bacteria	9	22	

Table 3. Differences in the temperature of the same anatomical areas on adjacent extremities in 3 different cows

Number	Anatomical region	The difference in temperament with the neighboring limb measured by a thermal imaging camera	Clinical findings – methods of inspection and palpation	Differential diagnosis
1. Fig. 3	Tarsal joint of right limb	2,6 °C	Moderate swelling on the lateral and medial side of the tarsal joint, with present skin damage.	Tarsal arthritis and tarsal hygroma
2. Fig. 4	Crown of hoof of front right leg	7,7 °C	Temperate swelling in the area of the crown of the hoof, palpation is extremely painful. Inability to lean/extremely difficult to lean on an extremity. When standing up, the animal transfers its entire weight to the adjacent limb.	Hoof arthritis, arthritis of the crown joint
3. Fig. 5	Carpal joint of left limb	1,1 °C	Painless swelling with the inability to manually determine the difference in temperature between adjacent extremities. Fluctuation phenomenon present.	Carpal arthritis and carpal hygroma

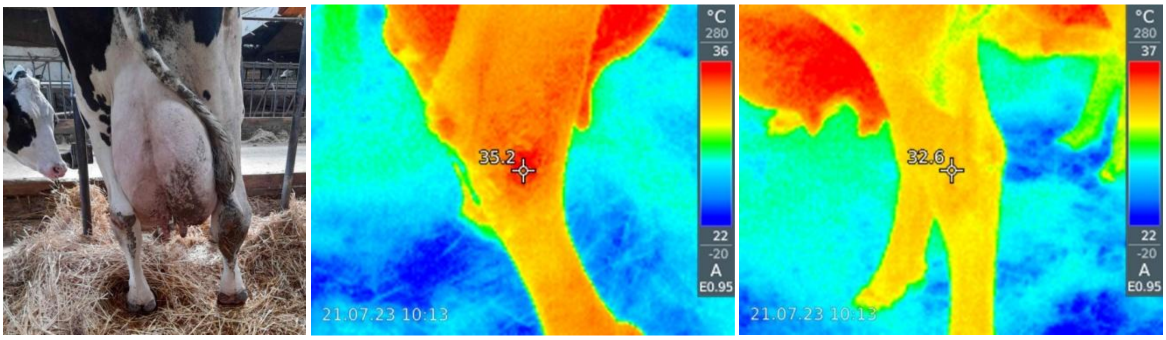

Figure 3. Tarsal arthritis – presumptive diagnosis

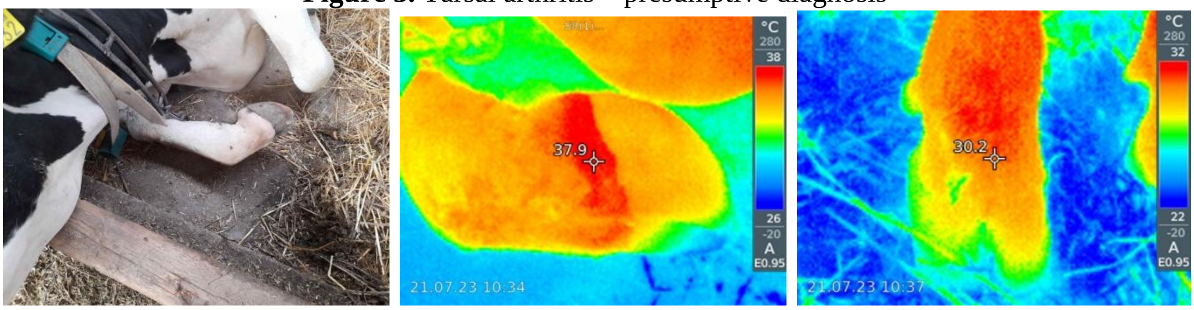

Figure 4. Swelling in region of crown of hoof of front right leg

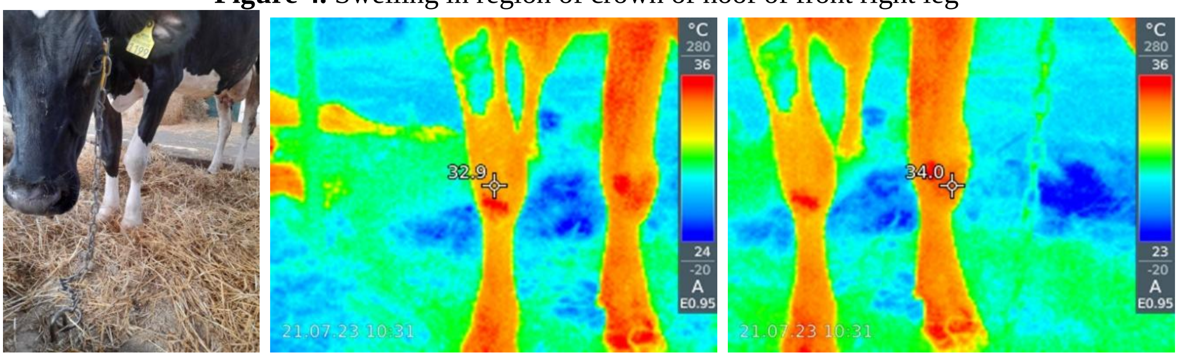

Figure 5. Swelling in region of carpal joint of left limb

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DISCUSSION

Ultrasonography is non-invasive diagnostic tool, ideal for evaluation of musculoskeletal disorders and diseases such as arthritis, bursitis, hygromas and abscesses, because these diseases are frequently associated with extensive soft tissue swelling and presence inflammatory exudation (1,2).

Ultrasound examination can provide information about the type, nature, and duration of some inflammatory processes, and these information can be helpful in planning therapy protocols for mentioned diseases (1,2,14,15).

In recent studies (1,2,6-8,16), it was reported that evaluation of the echogenicity of effusions and flow phenomena in some musculoskeletal disorders and diseases, which ranges from anechoic to echoic, depend on the type and nature of its inflammatory content (serous, serofibrinous, fibrinous, purulent). In the study with cows (15), it was reported that capsule echogenicity instead of capsule thickness is a more reliable ultrasonographic parameter for determination of the duration of the inflammatory process.

It has been also mentioned that ultrasonography cannot definitely characterize the composition and type of effusion content by itself. It was recommended to perform centesis and laboratory analysis of aspirated fluid samples (1,2,5-8). Rohde et al. (17), defined some laboratory musculoskeletal disorders and diseases in cattle which are associated with extensive soft tissue swelling and inflammatory exudation. Spasojevic et al. (18) found that could be possible to correlate ME values with laboratory parameters such as concentrations of Tp and uric acid, specific weight and percentage of Ne.

Our results could be used for confirmation of clinical cases of effusions in cattle and evaluation of therapy effects. That means that those patients with higher ME values could be in higher risks to have effusions with septic process in it, and according to that, it could define a therapy protocol, evaluation and prognosis of a diagnosed diseases.

Thermography, as other clinical method used in this study, finds its application primarily in the diagnosis of diseases of the locomotor system (primarily diseases of the distal parts of the extremities - arthritis and diseases of the hoofs), then in the diagnosis of diseases of the udder and skin (10-13,19).

What is important to emphasize when performing thermography in the diagnosis of arthritis is that thermography is performed after the general part of the clinical examination (which first of all involves the performance of inspection and palpation), and before the performance of other, special methods of clinical examination, and that it is necessary to perform imaging of the same anatomical areas of adjacent limbs because the diagnosis is based on the temperature difference of the obtained results (11-13). In our study, thermography was also performed after the general part of the clinical examination. Different studies have shown that differences in temperature values of 0.5 to 1.5 °C between the affected and unaffected areas on the adjacent extremities, speak in favor of the existence of inflammatory processes, especially if there are also various clinical signs that showed existence of inflammatory processes (11,13). In our study, differences in temperature between affected and unaffected structures were significant (*Table 3*) and indicated the existence of inflammatory processes. The non-invasiveness of thermography, then the detection of minimal temperature variations by comparing thermograms of identical structures of neighboring extremities, makes thermography one of the most useful methods in early presumptive diagnosis, when changes in microcirculation and an increase in temperature of the affected structures occur, rather than clinical manifestations of the disease (10-13).

CONCLUSION

According to the results of this study, it could be concluded that IRT is more useful and faster diagnostic tool in making early presumptive diagnosis. IRT can reveal anatomical, topographic and functional changes according to changes in microcirculation, but not the exact anatomical localization of the processes themselves, nor their closer characterization. Ultrasound examination is more suitable and useful diagnostic tool which can provide more exact localization and characterization of some inflammatory processes and their content, especially processes characterized by swelling and presence of some bigger amount of inflammatory exudate.

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LITERATURE

1. *Kofler J. Ultrasonography as a diagnostic aid in bovine musculoskeletal disorders. Vet Clin North Am Food Anim Pract. 2009, 25 (3): 687-731.*
2. *Kofler J., Geissbühler U., Steiner A. Diagnostic imaging in bovine orthopedics. Vet Clin North Am Food Anim Pract. 2014, 30 (1): 11-53.*
3. *Spasojević J. Evaluacija ultrazvučnog, citološkog, biohemijskog i mikrobiološkog pregleda u dijagnostici i determinaciji eksudativnih oboljenja lokomotornog sistema goveda. Doktorska disertacija. Univerzitet u Novom Sadu, Poljoprivredni fakultet. 2019.*
4. *Kofler J. Ultrasonographic examination of the carpal region in cattle-normal appearance. Vet J. 2000, 159 (1): 85-96.*
5. *Kofler J., Edinger H.K. Diagnostic ultrasound imaging of soft tissues in the bovine distal limb. Vet Radiol Ultrasound. 1995, 36 (3): 246-252.*
6. *Heppelmann M., Rehage J., Kofler J., Starke A. Ultrasonographic diagnosis of septic arthritis of the distal interphalangeal joint in cattle. Vet J. 2009, 179 (3): 407-416.*
7. *Kofler J. Arthrosonography - The use of diagnostic ultrasound in septic and traumatic arthritis in cattle - A retrospective study of 25 patients. Br Vet J. 1996, 152 (6): 683-698.*
8. *Kofler J, Altenbrunner-Martinek B. Ultrasonographic findings of disorders of the tarsal region in 97 cattle-arthritis, bursitis, tenosynovitis, periarticular abscess and vein thrombosis. Berl Munch Tierarztl Wochenschr. 2007, 121 (3-4): 145-158.*
9. *Kofler J., Martinek B. Ultrasonographic imaging of disorders of the carpal region in 42 cattle-arthritis, tenosynovitis, precarpal hygroma, periarticular abscess. Tierarztl Prax. 2004, 32 (2): 61-74.*
10. *Racewicz P., Sobek J., Majewski M., Różańska-Zawieja J. The use of thermal imaging measurements in dairy cow herds. Animal Science and Genetics. 2018, 14(1): 55-69.*
11. *Arican M., Erol H., Altan S., Köylü Ö. The use of infrared thermography in the early diagnosis of septic arthritis in calves. Bulg J Vet Med. 2022, 25(2): 298-307.*
12. *Yanmaz L.E., Dogan E., Okumus Z., Mahir K., Senocak M.G., Cengiz S. Radiographic, ultrasonographic and thermographic findings in neonatal calves with septic arthritis: 82 cases (2006-2013). Ataturk Univ. Vet. Bilim. Derg. 2016, 11(1): 6-12.*
13. *Alsaaod M., Schaefer A.L., Büscher W., Steiner A. The role of infrared thermography as a non-invasive tool for the detection of lameness in cattle. Sensors. 2015, 15(6): 14513-14525.*
14. *Abouelnasr K., El-Shafaey E.S., Mosbah E., El-Khodery S. Utility of ultrasonography for diagnosis of superficial swellings in buffalo (Bubalus bubalis). J Vet Med Sci. 2016, 78 (8): 1303-1309.*
15. *Seyrek-Intas D., Celimli N., Gorgul O.S., Cecen G. Comparison of clinical, ultrasonographic, and postoperative macroscopic findings in cows with bursitis. Vet Radiol Ultrasound. 2005, 46 (2): 143-145.*
16. *Magda M.A., Abdel-Hakiem M.A.H. Ultrasonographic differential diagnosis of superficial swellings in farm animals. J Adv Vet Res. 2012, 2 (4): 292-298.*
17. *Rohde C., Anderson D.E., Desrochers A., St-Jean G., Hull B.L., Rings D.M. Synovial fluid analysis in cattle: A review of 130 cases. Vet Surg. 2000, 29 (4): 341-346.*
18. *Spasojevic J., Toholj B., Cincovic M., Stevancevic M., Belic B., Kos J. The Correlations Between Mean Echogenicity and Laboratory Findings of Superficial Swellings in 50 Dairy Cows. Kafkas Univ Vet Fak Derg 2018; 24(4): 545-550.*
19. *Džermeikaitė K., Bačėninaitė D., Antanaitis, R. Innovations in Cattle Farming: Application of Innovative Technologies and Sensors in the Diagnosis of Diseases. Animals 2023, 13(5):780.*

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UTICAJ PERIODA LAKTACIJE I ZDRAVLJA NA VREDNOST PARAMETARA U ZBIRNOM UZORKU KRVI

THE INFLUENCE OF THE LACTATION PERIOD AND HEALTH ON THE VALUE OF THE PARAMETERS IN THE PULLED BLOOD SAMPLE

Nenad Staničkov[a*], Mira Majkić[b], Marko Cincović[b], Radojica Djoković[c], Miloš Ži. Petrović[c], Sandra Nikolić[b], Siniša Grubač[d]

[a]Srednja poljoprivredno-prehrambena škola Sombor, Srbija
[b]Departman za veterinarsku medicinu, Poljoprivredni fakultet, Novi Sad, Srbija
[c]Agronomski fakultet Čačak, Univerzitet u Kragujevcu, Srbija
[d]Naučni institut za veterinarstvo Novi Sad, Srbija
*Autor za kontakt: dr Nenad Staničkov, nenadstanickov@gmail.com

M.M. https://orcid.org/0009-0007-0110-7327, M.C. https://orcid.org/0000-0002-5683-8983, R.Dj. https://orcid.org/0000-0003-0900-3227, M.Ži.P. https://orcid.org/0000-0002-4707-6796, S.N. https://orcid.org/0000-0001-7337-9937, S.G. https://orcid.org/0000-0002-8967-1358

SAŽETAK

Zbirni uzorci krvi predstavljaju ekonomičan i kvalitetan način da se prikaže stanje određene populacije, kao i odgovor populacije na određene faktore spoljašnje i unutrašnje sredine, upotreba zbirnih uzoraka krvi u veterinarskoj medicini i srodnim oblastima našla je široku primenu, bilo da se radi o dijagnostici zaraznih bolesti, upotrebi uzoraka u internoj kontroli kvaliteta u laboratoriji ili u dijagnostici različitih bolesti. Pravljenje zbirnog uzorka krvi štedi vreme i redukuje cenu analiza, a omogućuje i lakše čuvanje uzorka, dok kod pojedinačnog uzorka trebaju veći prostorni, vremenski i finansijski resursi, uz veći izvor grešaka. U analitičkom smislu veća zapremina zbirnog uzorka (sačinjena od male zapremine većeg broja uzoraka) krvi omogućuje bolje određivanje limita detekcije, dok se u slučaju pojedinačnih uzoraka mora obezbediti dovoljno velika količina tog jednog uzorka. Statističko procenjivajne se takođe razlikuje, tako da kod zbirnog uzorka krvi možemo proceniti srednju vrednosti, ali ne i varijabilnost kao u populaciji pojedinačnih uzoraka. Kao opšte pravilo, srednja vrednost prikupljenih uzoraka treba da bude blizu sredine referentnog opsega da bi se smatrala normalnim. Kriterijumi za tumačenje srednjih vrednosti nisu do kraja definisani. Smatra se da bi dobijena srednja vrednost trebala da budu u rasponu od 1 ili 1,3 standardne devijacije u referentnoj populaciji kako bi mgli da zaključimo da nema jedinki sa značajnim metaboličkim problemima. Nađeno je da ukoliko ima do 10% abnormalnih metaboličkih vrednosti u populaciji imaćemo malo odstupanje vrednosti pula od srednje vrednosti populacije koje će iznositi oko 0,26 standardnih devijacija, ali da to u velikoj meri zavisi od analita koji se meri. Naši rezultati su pokazali da prosečna vrednost pojedinačnih uzoraka krvi i koncentracija dobijena pulovanjem tih pojedinačnih uzoraka daje gotovo identične vrednosti koje se identično menjaju u funkciji perioda laktacije i zdravlja krava. Zbog svega navedenog spravljanje pula uzorka može biti zanimljiv alat u oceni metaboličkog profila na većim populacijama krava.

Ključne reči: krave, metabolički profil, zbirni uzorak krvi.

ABSTRACT

Pooled blood samples are an economical and high-quality way to show the state of a certain population, as well as the response of the population to certain factors of the external and internal environment, the use of pooled blood samples in veterinary medicine and related fields has found wide application, whether it is for the diagnosis of infectious diseases , the use of samples in internal quality control in the laboratory or in the diagnosis of various diseases. Creating a pooled blood sample saves time and reduces the cost of analysis, and enables easier storage of the sample, while individual samples require greater space, time and financial resources, with a greater source of errors. In the analytical sense, a larger volume of the collective sample (made up of a small volume of a larger number of samples) of blood enables a better determination of the detection limit, while in the case of individual samples, a sufficiently large amount of that one sample must be provided. Statistically estimable also differs, so with a pooled blood sample we can estimate the mean, but not the variability as in a population of individual samples. As a general rule, the mean value of the collected samples should be close to the middle of the reference range to be considered normal. The criteria for the interpretation of mean values have not been fully defined. It is considered that the mean value obtained should be in the range of 1 or 1.3 standard deviations in the reference population in order to be able to conclude that there are no individuals with significant metabolic problems. It was found that if there are up to 10% of abnormal metabolic values in the population, we will have a small deviation of the pool value from the population mean, which will amount to about 0.26 standard deviations, but that it largely depends on the analyte being measured. Our results showed that the average value of individual blood samples and the concentration obtained by pooling those individual samples give almost identical values that change identically as a function of the lactation period and the health of the cows. Due to all of the above, the preparation of pooled samples can be an interesting tool in the evaluation of the metabolic profile of larger cow populations.

Key words: cows, metabolic profile, pooled blood sample.

ZBIRNI UZORCI KRVI I PRAKTIČNE SMERNICE ZA FORMIRANJE I INTERPRETACIJU PULEVA KRVNIH SERUMA

Zbirni uzorci krvi predstavljaju ekonomičan i kvalitetan način da se prikaže stanje određene populacije, kao i odgovor populacije na određene faktore spoljašnje i unutrašnje sredine, upotreba zbirnih uzoraka krvi u veterinarskoj medicini i srodnim oblastima našla je široku primenu, bilo da se radi o dijagnostici zaraznih bolesti, upotrebi uzoraka u internoj kontroli kvaliteta u laboratoriji ili u dijagnostici različitih bolesti. Potrebno je poznavati specifičnosti rada sa zbirnim uzorcima uključujuću i prednosti i njihova ograničenja (1-6). Pravljenje zbirnog uzorka krvi štedi vreme i redukuje cenu analiza, a omogućuje i lakše čuvanje uzorka, dok kod pojedinačnog uzorka trebaju veći prostorni, vremenski i finansijski resursi, uz veći izvor grešaka. U analitičkom smislu veća zapremina zbirnog uzorka (sačinjena od male zapremine većeg broja uzoraka) krvi omogućuje bolje određivanje limita detekcije, dok se u slučaju pojedinačnih uzoraka mora obezbediti dovoljno velika količina tog jednog uzorka. Statističko procenjivajne se takođe razlikuje, tako da kod zbirnog uzorka krvi možemo proceniti srednju vrednosti, ali ne i varijabilnost kao u populaciji pojedinačnih uzoraka. Potrebno je poznavati osobine uzoraka koji se puluju, kao i parametara koji se u njima mere. Određivanje biomarkera iz zbirnog uzorka krvi može se sprovoditi u zavisnosti od ciljeva istraživanja i praktičnog značaja istraživanja. Zbirni uzorci krvi su vrlo korisni za procenu relativnih nivoa izloženosti među podpopulacijama ili za praćenje trendova u nivoima tokom vremena ili prostora. Zbirni uzorci krvi podrazumevaju da se određena količina većeg broja uzoraka krvi sabere u jedinstven uzorak i da se određeni parametar meri u jednom zbirnom uzorku, a ne u mnogo pojedinačnih. Zbog prirodne težnje određenih vrednosti da se kreću oko svoje srednje vrednosti, kao mere centralne tendecije (zakon usrednjavanja), zbirni uzorak krvi pokazuje vrednost koja odgovara aritmetičkoj sredini populacije, odnosno aritmetičkoj sredini pojedinačnih uzoraka koji ulaze u sastav zbirnog uzorka krvi. Naši rezultati, bilo da se radi o izmerenim vrednostima ili da se radi o pozicionim vrednostima (Z skor), pokazuju upavo ovu zakonitost. Takođe, poznato je da ako je koncentracija biomarkera normalno raspoređena u populaciji, tada će izmerene koncentracije u više zbirnnih uzoraka uzetih iz te populacije takođe biti normalno raspoređene sa istom srednjom vrednošću. Pod idealnim uslovima, i uz jednostavne pretpostavke o normalnosti osnovne distribucije populacije, varijansa populacije može se proceniti na osnovu varijanse u izmerenim koncentracijama u više nezavisno konstituisanih grupa. Međutim, primenu ovog idealnog koncepta ometaju tri faktora: doprinos greške merenja varijacijama u merenjima

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objedinjenih uzoraka; doprinos greške objedinjavanja varijacijama merenja objedinjenih uzoraka; i oblik osnovne populacijske distribucije koncentracija biomarkera, koje često ne zauzimaju normalnu distribuciju. Samo prikupljanje uzoraka mogu uvesti poseban i nezavisan izvor greške u analizu, što se naziva „greška u grupisanju“. Greška prikupljanja može biti posebno relevantna za zbirne uzorke koji se sastoje od velikog broja pojedinačnih uzoraka, pri čemu je greška aditivna. Greška u grupisanju proizilazi iz fizičkih grešaka povezanih sa izdvajanjem i prenosom preciznih količina iz više pojedinačnih uzoraka u fizički skup. Greške povezane sa ovim procedurama mogu se pojačati ako se postupak udruživanja oslanja na šemu ponderisanja. Tako je predložena šema objedinjavanja u kojoj je obim uzorka od svakog pojedinca uključenog u zbir proporcionalan statističkoj težini uzorka populacije koja je dodeljena toj osobi. U takvom slučaju, slučajna greška u fizičkom procesu udruživanja može biti uvećana ili izobličena jer će greška u ekstrahovanju ili sastavljanju uzoraka od pojedinaca sa visokom težinom uticati na ukupnu grešku skupa u većoj meri. Kvantifikovanje i karakterizacija ovog izvora greške može zahtevati fizičko eksperimentisanje, gde se proces udruživanja replicira na osnovne rastvore kako bi se procenila relativna veličina ovog izvora greške u poređenju sa drugim potencijalnim izvorima, kao što je greška merenja. Poznati su i statistički pristupe za procenu varijanse populacije i specifičnih percentila na osnovu analize objedinjenih uzoraka. Pristup se oslanja na na tri značajna elementa i to su pretpostavku lognormalnosti u osnovnim podacima; analiza višestrukih grupa da bi se omogućila procena varijanse populacije; i ponovljena analitička određivanja svakog skupa kako bi se omogućila procena varijanse od greške merenja. Na osnovu uočenih varijacija u grupnim merenjima i procene greške merenja, može se proceniti varijansa osnovne populacije. Pouzdanje u procenu osnovne varijanse stanovništva raste sa povećanjem broja grupa iz datih slojeva stanovništva. Slično, tačnost procene greške merenja raste sa povećanjem broja ponovljenih analitičkih određivanja po grupi. Ove procene varijanse u okviru grupe i unutar grupe mogu se kombinovati sa ponderisanom aritmetičkom sredinom izmerenih koncentracija u grupi da bi se procenila i geometrijska sredina i specificirani percentili u populaciji, opet pod pretpostavkom log-normalnosti za distribuciju populacije. Međutim, u meri u kojoj osnovna distribucija stanovništva odstupa od log normalnosti, ovi pristupi neće pružiti tačne procene varijanse ili distribucije populacije. Zbog svega navedenog je od velikog značaja bilo da se utvrdi oblik i karakteristike distribucije frekvencije zbirnih uzoraka krvi u našem ogledu, gde je precizno utvrđeno koji parametri zauzimaju normalnu raspodelu, a koji parametri ne zauzimaju ovakvu raspodelu. Poređenjem distribucija frekvencija zbirnih uzoraka krvi u odnosu na distribucije pojedinačnih uzoraka krvi zaključujemo da su zbirni uzorci krvi pokazali određeni stepen normalizacije, što se slaže sa predhodno objašnjenim pravilom da zbirni uzorci pokazuju određeni stepen uprosečavanja i sklonosti da dobijene vrednosti idu prema opštoj srednjoj vrednosti kao glavnom pokazatelju centralne tendecije. Kada se izrađuje metabolički profil kod krava on podrazumeva da se određeni broj pojedinačnih krava iz različitih perioda laktacije procenjuje najčešće prostim poređenjem dobijenih vrednosti sareferentnim vrednostima. To mogu biti opšte prihvaćene referentne vrednosti koje su predhodno validirane ili referentne vrednosti zasnovane na stadu u kom se vrši metaboličko ispitivanje, što bi zapravo bila idealna situacija. Međutim, u određenim okolnostima kada se koristi zbirni uzorak krvi tada se dobija jedinstvena vrednost grupe pa nemamo nikakvih podataka o varijabilnosti. U tom smislu se smatra da je potrebno formirati i referentne vrednosti za pul uzoraka kako bi se adekvatno poredili rezultati pula sa referentnim vrednostima pula, dok prosto poređenje sa referentnim vrednostima za pojedinačne životnje može da dovede do pogrešne klasifikacije i netačne interpretacije zdravstvenog i produktivnog statusa krava. Kod tumačenja prosečnih vrednosti odnosno kod zbirnog uzorka krvi nije dovoljno da se uporedi da li je dobijena vrednost u okviru ili zvan referentnih već je mnogo pravilnije da se poredi dobijena vrednost i njeno variranje u odnosu na centralnu tendeniciju referentne populacije, tj. da se poredi udaljenost vrednosti dobijene u pulu od srednje vrednosti referentne populacije. Kada se tumače objedinjeni uzorci, treba imati na umu da izmerena vrednost predstavlja populaciju sa pojedincima iznad i ispod srednje vrednosti. Kao opšte pravilo, srednja vrednost prikupljenih uzoraka treba da bude blizu sredine referentnog opsega da bi se smatrala normalnim. Kriterijumi za tumačenje srednjih vrednosti nisu do kraja definisani. Smatra se da bi dobijena srednja vrednost trebala da budu u rasponu

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od 1 ili 1,3 standardne devijacije u referentnoj populaciji kako bi mgli da zaključimo da nema jedinki sa značajnim metaboličkim problemima. Nađeno je da ukoliko ima do 10% abnormalnih metaboličkih vrednosti u populaciji imaćemo malo odstupanje vrednosti pula od srednje vrednosti populacije koje će iznositi oko 0,26 standardnih devijacija, ali da to u velikoj meri zavisi od analita koji se meri. Tako je nađeno da devijacija bila u rasponu od od 0,11 za glukozu do 0,6 standardnih devijacija za BHB standardnih devijacija. Nađena je linearna povezanost između broja standardnih devijacija izmerena srednja vrednost ili zbirni uzorak koji se udaljio od srednje vrednosti referentne populacije (ili medijane) i procenta abnormalnih vrednosti unutar grupnog uzorka. Koristeći ove odnose, mogu se generisati smernice za koncentracije analita za interpretaciju srednjih ili zbirnih uzoraka. Ono što nije do sada utvrđeno je kako se ove udaljenosti menjaju u funckiji procenta obolelih krava od neke bolesti, pa je to bila jedna od tema ovog rada. Trenutno su dostupne smernice za tumačenje svežih uzoraka krava. Slične dijagnostičke interpretacije kao što je prethodno opisano mogu se postići sa različitim analitima izmerenim u srednjim ili zbirnim uzorcima. Tako je nađeno da ukoliko je koncentracija albumina u pulu 38,5 g/L nema pojedinačnih uzoraka sa abnormalnim vrednostima, a procentualno učešće abnormalnih uzoraka će biti 20% ako je koncentraciaj albumina 36,8, odnosno 40% ako je 35,1 g/L. Kada se radi o AST situacija je sledeća: 0% abnormalnih ako asto 93,8 IU/L, 20% abnormalnih ako je 99,3 IU/L i 40% abnormalnih uzoraka postojia ako ima 104,7 IU/L. Kod BHB nema abnormalnih uzoraka ako je njegova vrednost 0,52 mmol/L, njihovo učešće je na nivou 20% ukoliko je vrednost 0,9 mmol/L, odnosno 40% ako je vrednost 1,28 mmol/L.

UTICAJ PERIODA LAKTACIJE I ZDRAVSTVENOG STANJA NA KRETANJE PROSEČNE VREDNOSTI POJEDINAČNIH UZORAKA I KONCENTRACIJE DOBIJENE IZ PULA TIH UZORAKA

Upotrebom ANOVA analize smo ispitali razliku pozicione vrednosti (Z-skor) i originalne vrednosti metabolita u zbirnom uzorku krvi kao i dobijenoj aritmetičkoj sredini pojedinačnih uzoraka koji su ušli u pripadajući zbirni uzorak, a koji potiču od krava u ranoj laktaciji, srednjoj laktaciji i od krava koje pokazuju metaboličke disbalanse u vidu hiperketonemije. ANOVA analiza je pokazala da većina ispitivanih parametara ispoljava statistički značajnu razliku (nivo značajnosti $p<0{,}01$ ili veći) u funkciji perioda laktacije i zdravstvenog statusa. Gotovo identično su se ponašali zbirni uzorak krvi, poziciona vrednost zbirnog uzorka krvi, aritmetička sredina pojedinačnih uzoraka koji ulaze u pripadajući zbirni uzorak i poziciona vrednost aritmetičke sredine uzoraka. Jedini parametar koji nije pokaza znake variranja je ukupni protein u krvi (TPROT). Rezultati ANOVA analize su prikazani u Tabeli. Nađeno je značajno odstupanje vrednosti parametara u ketotičnoj grupi krava, dok odstupanje vrednosti metaboličkih parametara kod krava u ranoj i srednjoj laktaciji nije pokazalo statistički značajno odstupanje za najveći broj parametara.

Kada se interpretira vrednost iz zbirnog uzorka krvi ne možemo vršiti direktno poređenje dobijenog rezultata sa referentnom vrednošću za krave. Zbirni uzorak krvi ima jedinstvenu numeričku vrednostu koja je zapravo srednja vrednost svih pojedinačnih uzoraka, što znači da će pojedine krave imati vrednosti ispod te vrednosti, odnosno iznad te vrednosti. Ukoliko je srednja vrednost zbirnog uzorka krvi blizu srednje vrednosti referentnog intervala koji koristimo u laboratoriji, velija je verovatnoća da sve krave u pulu uzorka imaju normalne vrednosti. Međutim, odstupanje vrednosti pula uzorka od srednje referentne verdnosti ukazuej na to da postoje krave čije vrednosti odlaze u gornji ili donji ekstrem, povlačeći za sobom srednju vrednost odnosno izmerenu vrednost pula u istom pravcu. Sa druge strane ako je srednja vrednost odnosno koncentracia zbirnog uzorka na npr. donjoj referennoj vrednosti za kalcijum ili gornjoj referentneoj vrednosti za betahidroksibutirat možemo očekivati da barem 50% krava koje su deo pula ima hipokalcemiju odnosno hiperketonemiju jer se nalaze izvan referentne vrednosti. Međutim, potrebno je utvrditi u kojoj meri samo jedan uzorak u pulu koji zauziam neku ekstremnu vrednost može da poremeti srednju vrednost pula tako da dobijemo pogrešno tumačenje da je mnogo veći broj krava izvan referentnog opsega. Ovo zahteva veliku meta-analizu za koncentraciju metabolita kod krava u zdravlju i bolesti da bi se znalo koje su ti najviše i najniže vrednosti koje su u naučnim rezultatim zabeležene da se mogu sresti kod različitih poremećaja, a potom se mora izvršiti simulacija kako ekstremna vrednost u funkciji broja uzoraka u pulu učestvuje u vrednosti zbirnog uzorka krvi. U

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našem radu smo u kontrolisanim uslovima radili na povećanom procentu učešća krava sa ketozom u pulu od deset uzoraka, tako da je jedna do šest krava u pulu imala ketozu i pratili smo pozivione vrednosti zbirnog uzorka za ispitane parametre, koji su pokazalida se značajno menjaju ako ima više od 30% ketoznih krava. Dijagnostička vrednost zbirnog uzorka krvi zavisi od od varijabilnosti metabolita, tako da varijabilni metaboliti sa širokim opsegom srednjih vrednosti, kao što su recimo enzimi jetre pokazuju manju dijagnostičku vrednosti u odnosu na manje varijabilne parametre. Međutim, velika ekstremna odstupanja enzima kod različitih stanja čine enzime ipak dovoljno upotrebljivim za procenu pomoću zbirnog uzorka.

Prilikom analize dobijene vrednosti zbirnog uzorka krvi u obzir se mora uzeti udaljenost vrednost zbirnog uzorka krvi o srednje referentne vrednosti za tu populaciju krava. Udaljenost od srednje vrednosti u okviru referentne vrednosti zapravo predstavlja standardnu devijaciju, a udaljenost predstavlja broj standardnih devijacija od srednje referentne vrednosti. U našem radu smo se rukovodili ovom statističkom logikom. Međutim, mi smo u našem radu otišli korak dalje koristeći zapravo Z vrednost. Z vrednost je pozicioni pokazatelj koji je univerzalan i može se primeniti na bilo koji referentni opseg, odnosno distribuciju frekvencije, pa je zbog toga mnogo korisniji u odnosu na vezivanje za standardnu devijaciju. Takođe, kod primene standardne devijacije potrebno je da izračunamo njenu vrednost, idealno u populaciji iz koje potiču uzorci krvi krava, dok je Z vrednost univerzalan vrednost. U ovom radu je nađena linearna veza između Z skora i procenta krava sa ketozom, odnosno Z skora i originalne vrednosti zbirnog uzorka krvi. Nađeno je (7-12) da linearna veza između procenta abnormalnih vrednosti unutar grupe i broja standardnih devijacija različitih od srednje dobijene vrednosti postoji i specifična je za svaki analit, što se slaže sa našim rezultatima. Isti autori zaključuju da ako je vrednost zbirnog uzorka bila manja od 0,25 standardnih devijacija (opseg 0,1-0,5) od srednje vrednosti zdrave populacije, onda je < 10% pojedinačnih krava koje učestvuju u pulu imalo abnormalne vrednosti metaboličkih parametara krvi.

Kada se svi ovi podaci znaju potrebno je da se formiraju referentne vrednosti zbirnih uzoraka krvi za različite analite, kako bi se na osnovu te vrednosti iz zbirnog uzorka moglo utvrditi koji procenat krava ima vrednosti metabolita izvan rutinski korišćenih uobičajenih referentnih vrednosti za pojedinačne uzorke krvi krava. U našem radu smo utvrdili referente opsege Z skora koji uvrđuju da se određeni procenat ketoznih krava nalazi u zbirnom uzorku krvi. Ovakva klasifikacija je moguća i slaže se se ranijim rezultatima istraživača (13).

Vrednost Z-skora pulovanog uzorka i izračunate srednje vrednosti pojedinalnih uzoraka koji učestvuju u pulu međusobno visoko koreliraju (koeficijentom teterminacije preko 99%). Na osnovu navednog zaključujemo da se Z-skor pulovanog uzorka može koristiti u proceni metaboličkog statusa pojedinačnih krava čiji uzorci ulaze u zbirni uzorak u kom je merena koncentracija parametara. Upotrebom ANOVA analize smo ispitali razliku pozicione vrednosti (Z-skor) i originalne vrednosti metabolita u zbirnom uzorku krvi kao i dobijenoj aritmetičkoj sredini pojedinačnih uzoraka koji su ušli u pripadajući zbirni uzorak, a koji potiču od krava u ranoj laktaciji, srednjoj laktaciji i od krava koje pokazuju metaboličke disbalanse u vidu hiperketonemije. ANOVA analiza je pokazala da većina ispitivanih parametara ispoljava statistički značajnu razliku (nivo značajnosti $p<0,01$ ili veći) u funkciji perioda laktacije i zdravstvenog statusa. Gotovo identično su se ponašali zbirni uzorak krvi, poziciona vrednost zbirnog uzorka krvi, aritmetička sredina pojedinačnih uzoraka koji ulaze u pripadajući zbirni uzorak i poziciona vrednost aritmetičke sredine uzoraka. Nađeno je značajno odstupanje vrednosti parametara u ketotičnoj grupi krava, dok odstupanje vrednosti metaboličkih parametara kod krava u ranoj i srednjoj laktaciji nije pokazalo statistički značajno odstupanje za najveći broj parametara. Upotreba Z skora zbirnog uzorka krvi može biti korisna u proceni metaboličkog statusa krava, posebno kada postoje krave sa metaboličkim disbalansima. Potrebno je uzimati uže vremenske okvire u kojima se nalaze pojedinačni uzorci krava kada se vrši procena uticaja perioda laktacije na metaboličke parametre kako bi zbirni uzorak krvi pokazao da li postoje značajne razlike, zbog statističke osobine zbirnog uzorka da uprosečuje i stabilizuje vrednosti parametara koje u njemu merimo.

Naši rezultati zajedno sa rezultatima predhodnih istraživača (11-15) potvrđuju da se može uspešno koristiti zbirni uzorak krvi u proceni metaboličkog statusa krva u različitim periodima laktacija, ali da je potrebno usvojiti određena pravila pulovanja i grupisanja jedinki.

Grafikon 1. Sredjna vrednost pulovanih zbirnih uzoraka u ranoj (1) i srednjoj (2) laktaciji i kod krava u ketozi (3).

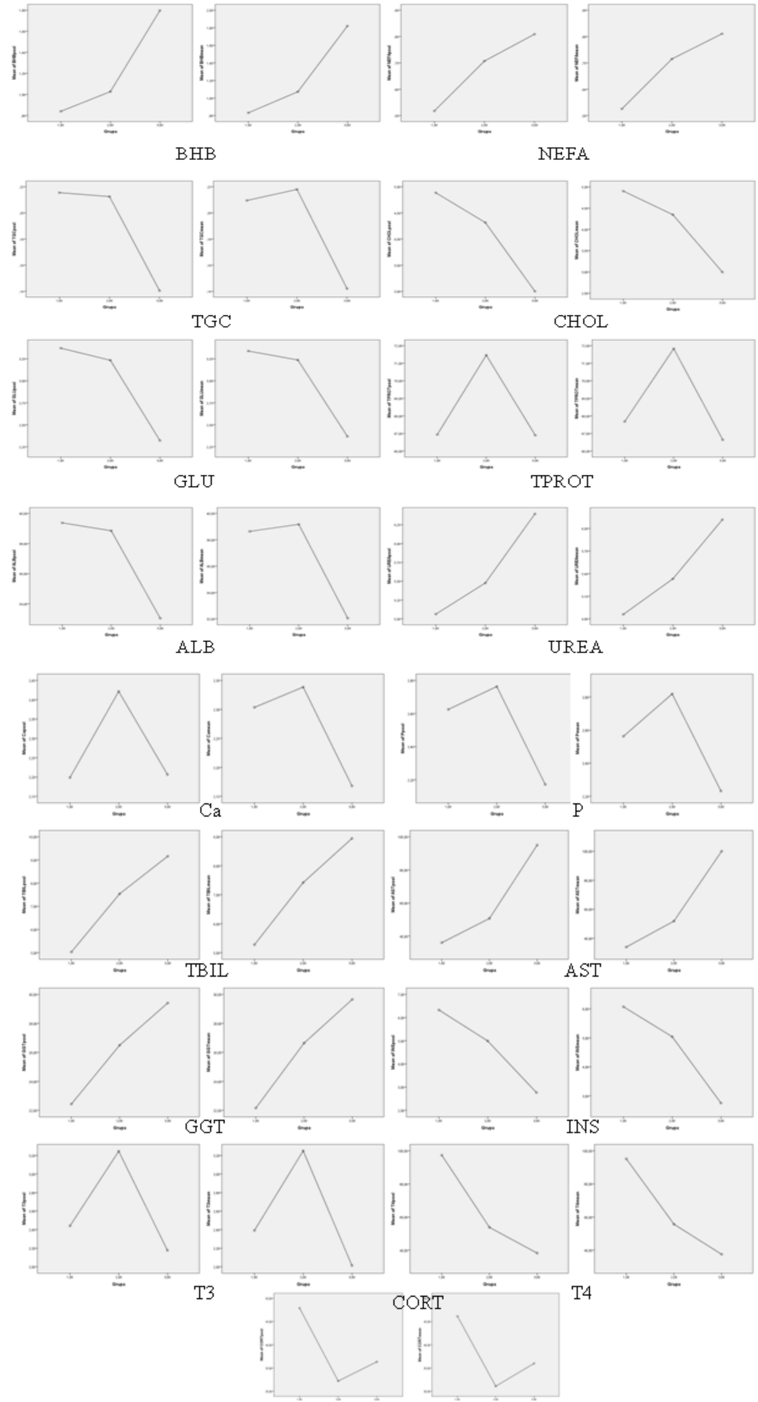

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Tabela 1. ANOVA analiza uticaj perioda laktacije i zdravstvenog statusa krava na vrednost parametara u zbirnom uzorku krvi i izračunatoj srednoj vrednosti pojedinačnih uzorakaka koji su ušli u navedeni pul

	Sum of Squares	Mean Square	F	p
BHBpool	10,280	5,140	124,102	**0,000**
BHBmean	10,633	5,317	138,056	**0,000**
ZBHBpool	122,247	61,124	123,923	**0,000**
ZBHBmean	126,868	63,434	136,944	**0,000**
NEFApool	,875	,438	11,885	**0,000**
NEFAmean	,842	,421	10,753	**0,000**
ZNEFApool	16,552	8,276	11,913	**0,000**
ZNEFAmean	15,958	7,979	10,758	**0,000**
TGCpool	,072	,036	5,479	**0,007**
TGCmean	,069	,035	5,152	**0,009**
ZTGCpool	7,212	3,606	5,479	**0,007**
ZTGCmean	6,928	3,464	5,164	**0,009**
CHOLpool	37,399	18,699	9,261	**0,000**
CHOLmean	38,457	19,228	9,437	**0,000**
ZCHOLpool	13,397	6,698	9,256	**0,000**
ZCHOLmean	13,839	6,920	9,459	**0,000**
GLUpool	13,066	6,533	19,543	**0,000**
GLUmean	11,414	5,707	17,211	**0,000**
ZGLUpool	22,602	11,301	19,471	**0,000**
ZGLUmean	19,776	9,888	17,143	**0,000**
TPROTpool	275,350	137,675	1,555	**0,220**
TPROTmean	301,725	150,863	1,543	**0,223**
ZTPROTpool	5,885	2,943	1,555	**0,220**
ZTPROTmean	6,449	3,225	1,543	**0,223**
ALBpool	495,236	247,618	8,086	**0,001**
ALBmean	630,496	315,248	10,452	**0,000**
ZALBpool	18,866	9,433	8,078	**0,001**
ZALBmean	24,085	12,043	10,463	**0,000**
UREApool	18,589	9,294	3,977	**0,024**
UREAmean	16,182	8,091	3,768	**0,029**
ZUREApool	9,724	4,862	3,963	**0,024**
ZUREAmean	8,492	4,246	3,770	**0,029**
Capool	,640	,320	6,812	**0,002**
Camean	,324	,162	3,236	**0,047**
ZCapool	6,676	3,338	6,845	**0,002**
ZCamean	3,412	1,706	3,285	**0,045**
Ppool	3,831	1,915	13,161	**0,000**
Pmean	3,458	1,729	11,555	**0,000**
ZPpool	23,967	11,983	13,130	**0,000**
ZPmean	21,636	10,818	11,573	**0,000**
TBILpool	173,593	86,797	10,168	**0,000**
TBILmean	135,828	67,914	8,354	**0,001**
ZTBILpool	3,840	1,920	10,123	**0,000**
ZTBILmean	3,032	1,516	8,382	**0,001**
ASTpool	37,660,786	18,830,393	30,211	**0,000**
ASTmean	46,851,808	23,425,904	34,795	**0,000**
ZASTpool	16,208	8,104	30,160	**0,000**
ZASTmean	20,118	10,059	34,733	**0,000**
GGTpool	491,425	245,712	7,316	**0,001**
GGTmean	569,067	284,534	8,082	**0,001**
ZGGTpool	6,614	3,307	7,303	**0,002**
ZGGTmean	7,685	3,843	8,060	**0,001**
INSpool	129,557	64,778	20,917	**0,000**
INSmean	115,765	57,883	19,080	**0,000**
ZINSpool	17,801	8,901	21,005	**0,000**
ZINSmean	15,861	7,930	19,004	**0,000**
T3pool	12,403	6,202	5,468	**0,007**
T3mean	16,063	8,032	7,256	**0,002**

ZT3pool	5,883	2,942	5,449	**0,007**
ZT3mean	7,620	3,810	7,264	**0,002**
T4pool	37,613,641	18,806,821	21,566	**0,000**
T4mean	34,788,742	17,394,371	20,534	**0,000**
ZT4pool	12,018	6,009	21,570	**0,000**
ZT4mean	11,067	5,534	20,429	**0,000**
CORTpool	2,650,159	1,325,080	6,341	**0,003**
CORTmean	2,359,365	1,179,682	6,420	**0,003**
ZCORTpool	6,560	3,280	6,341	**0,003**
ZCORTmean	5,840	2,920	6,420	**0,003**

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LITERATURA

1. *Heffernan A.L., Aylward L.L., Toms L.M.L., Sly P.D., Macleod M., Mueller J.F. Pooled biological specimens for human biomonitoring of environmental chemicals: opportunities and limitations. Journal of exposure science & environmental epidemiology 2014, 24(3), 225-232.*
2. *Caudill S.P. Characterizing populations of individuals using pooled samples. J Exposure Sci Environ Epidemiol 2010, 20: 29–37.*
3. *Schisterman E.F., Vexler A. To pool or not to pool, from whether to when: applications of pooling to biospecimens subject to a limit of detection. Paediatr Perinat Epidemiol 2008; 22: 486–496.*
4. *Schisterman E.F., Vexler A., Mumford S.L., Perkins N.J. Hybrid pooled-unpooled design for cost-efficient measurement of biomarkers. Stat Med 2010; 29: 597–613.*
5. *Caudill S.P. Use of pooled samples from the National Health and Nutrition Examination Survey. Stat Med 2012, 31: 3269–3277.*
6. *Guang Y., Yuzhong L., Hui L. Statistical and Experimental Evidence in a Design of Pooled Serum Sample Measurements to Improve Research Efficiency. Indian Journal of Pharmaceutical Sciences 2020, 21-25.*
7. *Van Saun R.J., Wustenberg M. Metabolic profiling to evaluate nutritional and disease status. Bov Pract 1997, 31(2): 37-42.*
8. *Van Saun R.J. Nutritional profiles: A new approach for dairy herds. Bov Pract 1997, 31(2): 43-50.*
9. *Van Saun R.J. Using a pooled sample technique for herd metabolic profile screening, in Proc 12th International Conf Production Diseases of Farm Animals, East Lansing, Michigan, July 18-23, pg 91 (Abstract P-9), 2004.*
10. *Van Saun R.J. Use and interpretation of pooled metabolic profiles for evaluating transition cow health status. Proc Am Assoc Bov Pract, 38:180, 2005.*
11. *Van Saun R.J. Validation of a pooled sample technique for herd metabolic profile screening. Proceedings XXIV World Buiatrics Congress, Nice, France, October 15-19, 2006a (Abstract #50/OS07-3 (CD-ROM Proceedings)).*
12. *Van Saun R.J. Interpretation of pooled metabolic profiles for evaluating transition cow health status. Proceedings XXIV World Buiatrics Congress, Nice, France, October 15-19, 2006b (Abstract #117/PS3-015 (CD-ROM Proceedings)).*
13. *Cincović M., Belić B., Toholj B., Stevančević M., Djokovic R.: Poboljšanje postupka procene metaboličkog statusa krava u ranoj laktaciji upotrebom zbirnog uzorka krvnog seruma–poboljšani tehnološki postupak M-84, 2015.*
14. *Lehwenich T. Investigation to the use of metabolic profile test in herd management of dairy cattle. Dissertation, Berlin University, Germany, 1999*
15. *Tornquist S.J., Van Saun R.J. Comparison of biochemical parameters in individual and pooled bovine sera. Vet Path 36(5):487, Abstr, 1990.*

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Review
Pregledni rad

DETERMINATION OF NEW BIOSECURITY INDICATORS ON CATTLE AND PIG FARMS BASED ON PUBLISHED STUDIES

UTVRĐIVANJE NOVIH INDIKATORA BIOSIGURNOSNOSTI NA GOVEDARSKIM I SVINJARSKIM FARMAMA NA OSNOVU OBJAVLJENIH STUDIJA

Branislav Stanković[a]*, Slavča Hristov[a], Marko Cincović[b], Radojica Djoković[c], Dušica Ostojić Andrić[d], Ivana Milošević-Stanković[a], Dimitar Nakov[e]

[a]University of Belgrade - Faculty of Agriculture, Belgrade-Zemun, Republic of Serbia
[b]University of Novi Sad - Faculty of Agriculture, Novi Sad, Republic of Serbia
[c]University of Kragujevac- Faculty of Agronomy, Čačak, Republic of Serbia
[d]Institute for Animal Husbandry, Belgrade – Zemun, Republic of Serbia
[e]Faculty of Agriculture, "Goce Delcev" University in Stip, Stip, Republic of North Macedonia
*Corresponding Author: baxton@agrif.bg.ac.rs

B.S. https://orcid.org/0000-0003-3925-6102, S.H. https://orcid.org/0000-0002-1719-9971, M.C. https://orcid.org/0000-0002-5683-8983, R.Dj. https://orcid.org/0000-0003-0900-3227, D.O.A. https://orcid.org/0000-0002-6913-4475, I.M.S. https://orcid.org/0000-0003-0448-4500, D.N. https://orcid.org/0000-0001-6159-5595

SUMMARY

In recent years, numerous papers have been published that consider indicators of cattle and pig farms biosecurity with the aim of improving it, and therefore their health and productivity. These indicators were presented in international projects published in indexed journals, as well as proceedings from international symposia. On-farm assessments usability of farm production biosecurity is generally well recognized. In the assessments of the farm biosecurity in different systems of rearing and accommodation, the need to determine new indicators in cattle and pig production was observed and discussed, based on a meta-analysis of previously published studies which include the most important papers in indexed journals and proceedings from international symposia that discussed the existing indicators. The analysis of the results of those studies will be used to determine the main characteristics of the existing new farm biosecurity indicators on cattle and pig farms with a focus on their applicability. The results, discussions and conclusions of those papers will be used to generate ideas to define biosecurity indicators on cattle and pig farms.

Key words: Biosecurity indicators, cattle farms, pig farms, literautre reivew

SAŽETAK

Poslednjih godina objavljeni su brojni radovi koji razmatraju indikatore biosigurnosti na farmi goveda i svinja u cilju njenog poboljšanja, a samim tim i njihovog zdravlja i produktivnosti. Ovi pokazatelji su predstavljeni u međunarodnim projektima objavljenim u indeksiranim časopisima, kao i u zbornicima radova sa međunarodnih simpozijuma. Procene farmske biosigurnosti u okviru proizvodnje je generalno dobro poznata. U procenama biosigurnosti farme u različitim sistemima uzgoja i smeštaja, uočena je i diskutovana potreba za određivanjem novih indikatora u govedarskoj i svinjarskoj proizvodnji, na osnovu ranije objavljenih studija koje obuhvataju najvažnije radove u indeksiranim časopisima i zbornicima radova sa međunarodnih simpozijuma koji su razmatrali postojeće indikatore. Analiza rezultata ovih studija biće korišćena za utvrđivanje glavnih karakteristika postojećih novih indikatora biobezbednosti farme na farmama goveda i svinja sa fokusom na njihovu primenljivost. Rezultati, diskusije i zaključci tih radova biće korišćeni za generisanje ideja za definisanje indikatora biosigurnosti na farmama goveda i svinja.

Ključne reči: indikatori biosigurnosti, farme krava, farme svinja, pregled literature

INTRODUCTION

Farm-level biosecurity consists of numerous working practices used to prevent minimize or at least control the penetration and spread of pathogens into a farm population, and its shed from the unit that may have an detrimental effect on the economy, environment and human health (1,2), when talking about farm level biosecurity, it should be kept in mind biosecurity plans, Hazard Analysis at Critical Control Points (HACCP), and risk management. Previously mentioned biosecurity plans have to prevention certain negative events (3), by undertaking suggested biosecurity measures at specific points of production process in the right moment (4), but how effective? Measuring success in biosafety and biosecurity activities in laboratories, Dickerson (5) concluded that there is a lack reliable data on the scope of number and types of laboratories, personnel "at risk”, activities conducted in labs, the effect of manipulations and technological innovations on biorisks, effectiveness of control measures, operational interventions on biosafety and biosecurity. In addition, there was a lack of standardized performance indicators and metrics, reliance on counting number of incidents to gauge "success", not to mention that there are no data on the impact of human behaviours on the effectiveness of any biorisk management systems, especially on pig and cattle farms. The aim of this paper is to suggest new, more appropriate and more useful biosecurity indicators in order to measure success of biosecurity measures during and after application of biosecurity plans.

DEFINITION OF TERM BIOSECURITY INDICATOR

Generally, an indicator is a focused, clear and specific characteristic which may determine or measure changes or progress of a program for achieving specific outcomes, at least one indicator for each outcome (6,7).
Any of the biosecurity indicators should be precise defined, in clear-cut terms that describe undoubtedly and accurately expected phenomenon, providing both qualitative and quantitative data and offer a simple and consistent approach to observe and evaluate achieved results (8). It is defined by Gudda (9) as CREAM: Clear, Relevant, Economic, Adequate and Monitorable, and also SMART: Specific, Measurable, Achievable, Relevant, and Time-bound (10).
In addition, according their traits, the indicators may be described in Table 1 (11):

Table 1. The biosecurity indicators by traits

Indicator type	Indicator description
Input indicators	Measuring the resources used, the amount of funding, time, or materials
Output indicators	Measuring the direct results of a program or project or set of measures undertaken
Outcome indicators	Measuring the changes or activity impacts results, improvements in health or income
Process indicators	Measuring level of a implemented set of measures, such as the provided services quality, the delivery rightness, or the level of stakeholder engagement
Impact indicators	Long-term, permanent effects of a programme or project can be measured, as specific type of performance indicator
Efficiency indicators	Measuring the cost-effectiveness of a program or project, such as the ratio of resources invested to results achieved
Effectiveness indicators	Measuring the amount to which the set of measures is achieving its objectives
Quality indicators	Measuring the quality of program or project outcome, such as the levels of beneficiaries
Sustainability indicators	Measuring the prospective for undertaken measures, program or project to continue after external maintain has finished

As it is given in table 1, the indicator has to be undoubtedly defined, quantifiable, and feasible within a realistic period, relevant to the objectives (11), so therefore valid (precise measure of a behaviour, practice or assignment that was undertaken), reliable (consistently measurable over time, but not subjective), precise (precisely defined), measurable (that can be proven by available methods), timely (measurable at relevant time intervals), programmatically important (achieving the programme objective), according to Gage and Dunn (12). All of the above says that biosecurity

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indicators survey needs to be performed periodically, in order to obtain more adequate, more reliable and more precise ones.

Basically, there two types of indicator that may be applied in biosecurity level or biorisck level assessment (10,13): quantitative indicators tell if the activities are taking place as it was planned, but do not provide any information on their effect or impact, and qualitative indicators are usually concerned with outcome, providing information on changes caused by the undertaken activities.

Previously stated indicate what is the good indicator of the level of biosecurity or biorisk on a livestock farm; when measuring or assessing achieved biosecurity level in certain moment of time on certain farm, there should be at least one indicator for one trait or outcome or result, which has to be focused, clear and specific, and precisely and unambiguously defined. When analyzing scientific papers and other type of publications, it is clear that farm biosecurity level assessment is based on defined biosecurity components: isolation, traffic control and sanitation (14), or pillars of biosecurity, that is physical protection, personnel management, material control and responsibility, transport and information security (15), more or less same way defined in detail (7).

SUGGESTED POTENTIAL FARM BIOSECURITY LEVEL INDICATORS

The inability to measure the biosecurity and hygiene level of farms precisely has been obstacle for a long time in the pursuit of improvements (7). If farm management should be encouraged to improve the biosecurity or hygiene status of their farm, it is essential for them to recognise and accept quantitative goals and benchmarks, which can be used to describe the farm with respect to its biosecurity level and hygiene status, so that the measures necessary for improvements can be identified and their impact subsequently measured, if possible quantitatively (16).

When analyzing available biosecurity documents and scientific papers, it is clear that an almost identical or very similar point of view of the problem and similar paths of measuring or assessment of biological risk or biosecurity level on livestock farms. Also, it has to be taken into account its complexity, that is whether it is easy to answer with yes or no or more or less; other consist of several parameters, each describing certain part of the issue (7).

In the paper published by Stanković et al. (7), several systems that have been created for inventories of biosecurity measures undertaken were mentioned, mostly developed as checklists or as manuals or as support material for vaccines, such as COMBAT system (Boehringer Ingelheim), helping to identify biosecurity hazards in PRRS infections in pig production. Many of these evaluating systems were developed with a view to controlling a specific disease, Wageningen University checklist developed for the risks factors and introduction and spread of Streptococcus suis in herds (16-18) and PADRAP system designed by the American Association of Swine Veterinarians (19) and Iowa State University (20), that assess the biosecurity protocols for rearing pig herds and identifying PRRSV infection potential risk factors.

The decision-making is a dynamic process, which helps to raise understanding biosecurity as applied concept by collecting and analyzing information, guiding their approach to biosecurity (21). Biosecurity awareness refers to a stakeholder's perception of specific information, government policy and safety principles; this aspect influences the on the whole anticipation and control of animal diseases (22). In the paper of Li at al. (23), stakeholders' understanding of biosecurity refers to their accepting of biosecurity policies, information, and values. As the most of biosecurity behaviours, the farmers' biosecurity consciousness level is the basis for adoption of biosecurity measures (24). Farmers' biosecurity awareness includes their understanding of methods for control of the farm biosecurity circumstances and their common sense responsibility for this. The improvement of farmers' awareness of biosecurity would guide the embodiment of biosecurity behaviours among farmers (25). Based on this, Li at al. (23) proposed the hypothesis that biosecurity awareness can encourage farmers to adopt biosecurity procedures, since knowledge of stakeholders, especially farmers, and their perception of biosecurity should be taken into account (26), analyzing not only biosecurity data, but the attitude of farmers towards biosecurity measures, and a detailed checklist of the biosecurity measures actually practiced on the farm as well. In general, farmers implemented measures to decrease contamination risks from humans and livestock other than pigs, but on the other hand, biosecurity measures related to replacement of animals were not

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applied often. They believe that the most important measures were the sanitary procedures appliance, a fence around the farm, the restriction of visits and vehicles, using bird-proof nets in windows, having changing facilities, applying quarantines, and the use of other measures related to replacement stock. Certain measure perception was significantly influenced by the procedures that are actually practiced on the farm; those who did not have a sanitary barriers insisted on the importance of vehicles disinfection, while those who had one did not. Finally, awareness of the relationships between perceptions and measures taken is important in creating useful pig farms biosecurity strategies. Nöremark et al. (27) investigated does relations exist between biosecurity routines and livestock species, geographic position and farm herd size were analysed. These authors discovered wide range in biosecurity routines application, both within and between groups, where certain farms had rather biosecurity high level. A higher level of biosecurity was related to farms with pigs only, compared to farms with cattle, sheep/goats or mixed species, and at larger farms vs. hobby farms. Noticed inconsistent biosecurity routines were interpreted due to a lack of knowledge of infections transmission; the farmers perceived the risk of introduction of disease as low, e.g. for the use of protective clothing by visiting professionals. More knowledge about the biosecurity routines and they variations among different farms may help to identify types of farms with higher risk for infectious disease introduction, as well as categories of high risk professionals who can spread infections between farms (28,29). Based on these data, there is opportunity to improve on-farm biosecurity, as well as to use of biosecurity routines application success on farms as important biosecurity indicator.

The sanitation and hygiene measures and biosecurity procedures in order to prevent and control infection to address antimicrobial resistance are primarily focused on human population protection, but they are also essential for public health, as they can decrease the emergence and spread of resistant bacteria. In this context, both hygiene and biosecurity procedures can be antimicrobial resistance-sensitive, e.g., improving use of clean water and sanitation facilities or supporting farmers to put into service biosecurity measures. These can be implemented at a system level through standard operative procedures (SOP), lessening risk factors embedded in social structures and address socioeconomic vulnerabilities. Correct use of antibiotics in therapy, antimicrobial resistance, the correctness, timeliness and increased use of sanitation preparations, or presence of persistent infections in farm populations might be used as quality indicator for successful sanitation procedures, especially if related SOP are not clear or followed (7).

According to Wayop et al. (18) antimicrobial resistance is described as a global threat to human and animal health, and therefore, one of the global objectives is antimicrobials use optimization in humans and animals (30). To achieve this, there is necessity to establish controlled approaches to optimize antimicrobial use in different animal production systems. In the Netherlands, for instance, the use of antimicrobials in animals was decreased significantly between 2009 and 2021 by 70.8% after the introduction of various regulations and measures (31), although a wide variation still exists among farmers and veterinarians in their level of antimicrobial use and prescription patterns (31, 32). For that reason, Royal Dutch Veterinary Association developed veterinary clinical practice guidelines. These guidelines are not obligatory, but they are part of a voluntary veterinary quality system, supporting veterinarians in their clinical decision-making, including antimicrobial prescribing practices (33), which offers a possibility for further antimicrobial use reduction, and, of course, it may be connected to the compromised farm biosecurity level.

Certain indicators might be unified for different purposes or types of questionnaires, like all types of farm contacts with contaminant sources, suggested by Brennan et al. (29), who report shortage of knowledge regarding the inter-farms types and frequencies of contact as pathogen transmission routes. These authors explored all types of contact and frequencies between cattle farms in a region, focusing on potential routes of pathogen transmission: sharing of equipment, humans and vehicles movement and contact over/through fences with neighbouring stock, wildlife and even wind (34,35). Information was obtained relating to contact types and frequencies, including those involving animal movements, equipment sharing between farms and any contractors or companies visiting the farms. These findings lead to better understanding of inter-farm contacts and may help to develop appropriate biosecurity and control practices, and to create mathematical modelling of infectious diseases.

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Similarly to the previous, the biomarkers of interest include animal-based measures, as indirect measurements of animal exposure to pathogens' presence and spread. The study of Scollo et al. (36) describes a novel biosecurity risk analysis tool - BEAT, along tailor-made biosecurity protocol and the survailance of biosecurity implementation to identify strong and weak points in pig farms production (37). The method incorporates both input and output parameters in order to assess the risks of introduction, exposure and spread of pathogen in intensive pig production. The output parameters are related to the biomarkers, such as animal-based indicators for continuous monitoring, and to give an early detection of breaches in biosecurity or biocontainment, such as:

Clinical Scores and Mortality, coughs and sneezing, and faeces on a 4-point scale (1 = firm and shaped; 2 = soft and shaped; 3 = loose; 4 = watery),

Slaughter Check, lesion scores on lungs, pleura, pericardium, and liver, as well as skin lesions on ear, tail, anterior and posterior of the carcass, with 3-point scale system (0 - up to one scratch or bite; 1 - from two to five scratches or bites, and score 2 - more than five scratches or bites, or any wound which penetrates the muscle), similar to the Welfare Quality® Protocol (38), and

Antimicrobial Use, method proposed by the EMA (39), in order to measure total antimicrobial use per year.

The final BEAT questionnaire include five sections related to external (entry risks in animal population) and internal (spread of pathogen between and in animal husbandry departments) biosecurity: the red zone (i.e., outside the farm perimeter, the public zone), the orange zone (the professional zone in-between the pigs' facilities), the green zone (the pigs' barns, the herd zone), and the two crossing points between external/professional zones (red/orange) and professional/internal zones (orange/green). Biosecurity and environmental sustainability were rated on a 4-point scale: a score of 0 was assigned to farms with completely inadequate biosecurity or sustainability practices, and a score of 3 was assigned to those with completely adequate biosecurity or sustainable practices (40). Therefore, biomarkers may bring important conclusions about level of farm biosecurity.

In addition, Chantziaras et al. (41) identified specific factors related to the environment, and discussed their relationship with health, welfare and reproductive performance in sows and piglets in different rearing systems, by: a) a questionnaire for farm management, interventions and housing, and b) farm production data related to the assorted performance parameters, such as litter index, replacement rate, repeat breeding, weaning to first mating interval and litter/piglet health performance: piglets born alive per litter, piglets born dead per litter, preweaning mortality rate and weaned piglets per litter. These factors are important for management and housing with significant effect on sow and piglet performances.

The Biocheck.UGent™ biosecurity risk-based scoring system for on-farm biosecurity quantification was developed at Ghent University for use in pig, poultry, beef and veal farms (42). It has general approach to biosecurity, focusing on paths of transmission of many types of transmissible diseases. Questionnaires for pig production include 109 (pig) mainly di- or trichotomous questions in several subcategories (2 to 19 questions each) for internal and external biosecurity, and weight factor for each subcategory and question, and the total score for internal and external biosecurity, ranged from 0 to 100 points (43-45).

Similar to mentioned Biocheck.UGent™, in 2011, the Ministry of Agriculture, Water Management and the Forestry of the Republic of Serbia financed the development of Guidances of Biosecurity Standards on cattle, pig and poultry farms (46-48), and the Questionary for farm biosecurity assessment within, related to numerous indicators, which was developed in Technological Research project 20110 "Welfare and Biosecurity Standards Development and Implementation in Improvement of Dairy and Pork Production" (2008-2011), and suported by Ministry of Science and Technology Development of Republic of Serbia (49). Each indicator with different numbers of parameters within, is rated from grade 0 to 5: Insufficient, without the potential to improve the biosecurity in the foreseeable future – 0; Insufficient, with the potential to improve the biosecurity in the foreseeable future – 1; Sufficient – 2; Good – 3; Very good – 4 and Excellent – 5, and summarized. In addition, a SWOT analysis (Strengths, Weaknesses, Opportunities, and Threats) takes place to get detailed impression of the possibilities for reducing the negative and improving the positive aspects of biosecurity on farms and completing the final audit. All of the 15 indicators have to be analyzed in order to find threats to biosecurity on the farm and overcome the

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disadvantages, risks that may hinder or prevent the overcoming disadvantages. The farm is then graded according to a rating scale: Group V 0-1.99 insufficient, Group IV 2.00-2.49 sufficient, Group III 2.5-3.49 good, Group II 3.5 - 4.49 very good and group 4.5 - 5.00 excellent. The indicators which are used are: 1. planning and monitoring the implementation of biosecurity measures, 2. farm isolation, 3. quarantine, 4. health status of the farm population, 5. movement and traffic control, 6. attitude towards visitors, 7. nutrition and water supply control, 8. manure management, 9. removal of dead animals, 10. Presence of other species of animals on the farm, 11. rodent population control, 12. Insect population control, 13. Bird control, 14. Sanitation, and 15. Farm's attitude towards the environment.

Comparing to Biocheck Pigs questionnaire, part A. farm characteristics, in Hristov & Stanković questionnaire (49,50), the size of the farm and categories of pigs are taken into account through different indicators, but the employment structure and size, their experience in keeping pigs, and the age of the facilities were not taken into account (7). In Hristov & Stanković questionnaire (50) "stand down" period was investigated, comparing to "pig-free period (more than 12 hours)" in Biocheck Pigs; part E. vermin and bird control are similar to indicators 11. Rodents control, 12. Insects control, and 13. Birds control; indicator 2. Farm isolation of presented questionnaire is similar to the part F. location of the farm of the Biocheck Pigs; differences are related to the wild boars presence; The indicator 4. Heard health status of the farm population is similar to the part G. Disease management of Biocheck Pigs. On the other hand, Biocheck Pigs parts H. Farrowing and suckling period and I. nursery unit and J. Finishing unit give more detail information regarding to this issue than in the presented questionnaire, which is covered by mentioned indicator; part K. measures between compartments, working lines and use of equipment of Biocheck Pigs is covered by different indicators of presented questionnaire (51).

The possibility to isolate farm or production unit and prevent physical breakthrough of vectors as indicator is often limited on perimeter and gate under control and should be supplemented with additional parameters. Hristov & Stanković (50) suggested that location of the premise in respect to and required distance from risk sources is necessary, as well as separation of clean and dirty routes for movement and supply on the farm, knowledge of dominant winds directions, and protective 'green' belt of trees and shrubs which surrounds the premises. Torremorell (52) pointed out that term 'bioexclusion', is required to prevent pathogen movement across protection zones, in order to eliminate or diminish the number of disease-causing organisms within the animal's environment.

According to Gröndal et al. (53), different perspectives on biosecurity can prevent or reduce misunderstandings between pig farmers and veterinarians. The study identified differences between the veterinarians and farmers and their perception of the biosecurity in Swedish pig herds. Taking into account mentioned differences and similarities of the different perspectives can help to improve communication and cooperation regarding biosecurity issues.

When assessing farm biosecurity, besides well chosen and designed indicators, a systematically created questionnaire is no less important. The questions should be related and, if necessary, partially intersect, which gives a clear and detailed picture of the situation on the farm. It is very demanding to create modular and systematic questionnaire which would give precise description of biosecurity level of particular farm, but when achieved, mentioned traits enables adjustment and increased usability of such questionnaire (7).

CONCLUSION

Presented data of available scientific papers indicate traits of the good farm biosecurity indicator. It is very complex issue, with high similarities of point of view of the problem and therefore there are similarities in measuring or assessment of biological risk or biosecurity level on livestock farms.

Some of them are simple, easy to answer with yes or no or more or less; other ones are complex, with several parameters within.

Generally, when measuring or assessing achieved biosecurity level in certain moment of time on certain farm, there should be obtained at least one indicator for one trait or outcome or result, focused, clear and specific, and precisely and unambiguously defined.

Survey of biosecurity indicators is complex and has to be performed periodically, in order to obtain more adequate, more reliable and more precise ones.

When assessing farm biosecurity, well chosen and designed indicators are required and placed in systematically created questionnaire. The questions should lean on each other and, if necessary partially overlap, and may give a clear and detailed picture of the situation on the farm. Modular and systematic questionnaire enables better adjustment and increased usability.

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REFERENCE

1. *Stanković B., Hristov S. Najčešći propusti u obezbeđenju biosigurnosti na farmama goveda i svinja. Zbornik naučnih radova, Agroekonomik 2009, 3-4, 103-110.*
2. *Stanković B., Hristov S., Zlatanović Z., Bojkovski J., Maksimović N. Sustainibility ahd Efficiency of Dairy Farms Biosecurity Plans. Agroznanje 2015, 16(4), 437-453.*
3. *Uhlenhoop E. Plan biosugurnosti na stočarskim farmama. Tematski zbornik "Dobrobit životinja i biosigurnost na farmama", 1. Međunarodna konferencija o dobrobiti i biosigurnosti na farmama u Srbiji, Poljoprivredni fakultet-Beograd, 227-238, 2007.*
4. *Stanković B., Hristov S., Petrujkić T., Marinković M., Blagojević M., Petrujkić B., Mirjana Todorović Joksimović, Vesna Davidović, Zlatanović Z. Biosigurnosne mere i standardi u proizvodnji semena nerastova na farmama i u centrima za reprodukciju. Zbornik kratkih sadržaja sa Simpozijuma Veterinarska medicina, stočarstvo i ekonomika u proizvodnji zdravstveno bezbedne hrane, Herceg Novi 24. juna -1. jula 2007, str. 47.*
5. *Dickerson B. Measuring Success in Biosafety and Biosecurity. https://www.osti.gov/servlets/purl/1645890, 2019 (accessed July 10, 2023).*
6. *Anon. Indicators. UN Women, 2010. https://www.endvawnow.org/en/articles/336-indicators.html (accessed July 10, 2023).*
7. *Stanković B., Hristov S., Cincović M., Đoković R., Ostojić Andrić D., Milošević-Stanković I., Nakov D. Determination of new biosecurity indicators on cattle and pig farms based on different publications. Proceedings of 14th International Symposium Modern Trends in Livestock Production, October 4-6th 2023, Belgrade, Serbia, 168-181, 2023.*
8. *Tengan C., Aigbavboa C., Thwala W. Monitoring and evaluation system and framework. 10.1201/9781003137979-3., 2021*
9. *Gudda P. A guide to project monitoring & evaluation. United States of America: AuthorHouse, 2011.*
10. *Anon. A compendium of indicators for monitoring and evaluating progress of the road map for neglected tropical diseases 2021–2030. Global Strategy. WHO. https://www.who.int/publications/i/item/9789240062863, 2023 (accessed July 10, 2023).*
11. *Anon. Measure your Success with Indicators in Monitoring and Evaluation. EvalCommunity. https://www.evalcommunity.com/career-center/indicators-in-monitoring-and-evaluation/, 2023 (accessed July 10, 2023).*
12. *Gage A., Dunn M. Monitoring and evaluating gender-based violence prevention and mitigation programs. Washington DC. Galtung, J: US Agency for International Development, Measure Evaluation, Interagency Gender Working Group, 2009.*
13. *Anon. Indicators. UN Women. https://www.endvawnow.org/en/articles/336-indicators.html, 2010 (accessed July 10, 2023).*
14. *Buchman M., Dewell G., Griffin D. Biosecurity Basics for Cattle Operations and Good Management Practices (GMP) for Controlling Infectious Diseases. G1411. Neb Guide. https://extensionpublications.unl.edu/assets/pdf/g1411.pdf, 2007 (accessed July 10, 2023).*
15. *Anon. Laboratory Biosecurity Controlling Laboratory Biorisks. SAND2012-4386C. https://www.osti.gov/servlets/purl/1117406, 2012 (accessed July 10, 2023).*
16. *Dewulf J. & Van Immerseel, F (Eds.). Biosecurity in animal production and veterinary medicine : from principles to practice. 1st Edition. Leuven, Belgium ; The Hague, The Netherlands: ACCO, 2018.*
17. *Wageningen University Checklist bestrijding Streptococcus suis door management-maatregelen. Wageningen University, Wageningen, the Netherlands, 2008.*
18. *Wayop I.Y., de Vet E., Wagenaar J.A., Speksnijder D.C. Why Veterinarians (Do Not) Adhere to the Clinical Practice Streptococcus suis in Weaned Pigs Guideline: A Qualitative Study. Antibiotics, 12(2), 320, 2023.*

19. *American Association of Swine Veterinarians – AASV. PADRAP (Production Animal Disease Risk Assessment Program), 2007.*
20. *Holtkamp D., Polson D., Wang C., Melody J. Quantifying risk and evaluating the relationship between external biosecurity factors and PPRS-negative herd survival. American Association of Swine Veterinarians, Omaha, Nebraska, USA, 2010.*
21. *Lin L., Liu Z., Du Y., Su S., Zheng Y. Psychological cognition of pollution prevention and control behavior of large-scale pig farmers and the effect of environmental regulation. Chin. J. Ecol. Agric. 2018, 26, 156–166.*
22. *Breen R., Karlson K.B., Holm A. Total, direct, and indirect effects in logit and probit models. Sociol. Methods Res. 2013, 42, 164–191. doi: 10.1177/0049124113494572.*
23. *Li J., Yuan M., Wang H., Zhou K. Government regulations, biosecurity awareness, and farmers' adoption of biosecurity measures: Evidence from pig farmers in Sichuan Province, China. Frontiers in Sustainable Food Systems, 7, 1106766, 2023.*
24. *Xu T., Chen H., Ji Y., Qiao D., Wang F. Understanding the differences in cultivated land protection behaviors between smallholders and professional farmers in Hainan Province, China. Front. Sustain. Food Syst 2023, 7, 1081671. doi: 10.3389/fsufs.2023.1081671.*
25. *Qiao J., Zhang X. The impact of government intervention and moral responsibility on the performance of aquaculture waste management: From the perspective of farm households. J. China Agric. Univ. 2019, 24, 248–259.*
26. *Casal J., De Manuel A., Mateu E., Martin M. Biosecurity measures on swine farms in Spain: perceptions by farmers and their relationship to current on-farm measures. Preventive veterinary medicine 2007, 82(1-2), 138-150.*
27. *Nöremark M., Frössling J., Lewerin S.S. Application of routines that contribute to on-farm biosecurity as reported by Swedish livestock farmers. Transboundary and emerging diseases 2010, 57(4), 225-236.*
28. *Boklund A., Toft N., Alban L., Uttenthal A. Comparing the epidemiological and economic effects of control strategies against classical swine fever in Denmark. Prev. Vet. Med. 2009, 90, 180–193.*
29. *Brennan M.L., Kemp R., Christley R.M. Direct and indirect contacts between cattle farms in north-west England. Prev. Vet. Med. 2008, 84, 242–260.*
30. *WHO. Global Action Plan on Antimicrobial Resistance; World Health Organization: Geneva, Switzerland, 2015, pp. 1–28. ISBN 9789241509763.*
31. *SDA. Autoriteit Diergeneesmiddelen. Het Gebruik van Antibiotica Bij Landbouwhuisdieren in 2021 (SDa-report ‘Usage of antibiotics in agricultural livestock in the Netherlands in 2021). Available online: https://www.autoriteitdiergeneesmiddelen.nl/nl/publicaties/sda-rapporten-antibioticumgebruik, 2021 (accessed on 3 July 2022).*
32. *Speksnijder D.C., Sanders P.M., Bens D.L.A., Meijboom F.L.B., Verheij T.J., Leneman J.M. Onderzoek Naar Kritische Succesfactoren voor een Laag Antibiotica Voorschrijfpatroon van Dierenartsen; Utrecht University: Utrecht, The Netherlands, 2020, pp. 1–128.*
33. *Speksnijder D.C., Mevius D.J., Bruschke C.J.M., Wagenaar J.A. Reduction of veterinary antimicrobial use in the Netherlands. The Dutch success model. Zoonoses Public Health 2015, 62 (Suppl. S1), 79–87.*
34. *Mikkelsen T., Alexandersen S., Astrup P., Champion H.J., Donaldson A.I., Dunkerley F.N., Gloster J., Sørensen J.H., Thykier-Nielsen S. Investigation of airborne foot-and-mouth disease virus transmission during low-wind conditions in the early phase of the UK 2001 epidemic. Atmospheric chemistry and physics 2003, 3(6), 2101-2110.*
35. *Woodroffe R., Donnelly C.A., Jenkins H.E., Johnston W.T., Cox D.R., Bourne F.J., Cheeseman C.L., Delahay R.J., Clifton-Hadley R.S., Gettinby G., Gilks P., Hewinson R.G., McInerney J.P., Morrison W.I. Culling and cattle controls influence tuberculosis risk for badgers. Proceedings of the National Academy of Sciences 2006, 103(40), 14713-14717.*
36. *Scollo A., Levallois P., Fourichon C., Motta A., Mannelli A., Lombardo F., Ferrari P. Monitoring Means and Results of Biosecurity in Pig Fattening Farms: Systematic Assessment of Measures in Place and Exploration of Biomarkers of Interest. Animals 2022, 12(19), 2655.*
37. *FAO. Animal Production and Health Division (NSA). Available online: https://www.fao.org/ag/againfo/programmes/en/empres/news_060815b.html, 2023 (accessed on August 31st 2023).*
38. *Bottacini M., Scollo A., Edwards S.A., Contiero B., Veloci M., Pace V., Gottardo F. (2018): Skin lesion monitoring at slaughter on heavy pigs (170 kg): Welfare indicators and ham defects. PLoS ONE, 13, e0207115.*
39. *EMA. Principles on Assignment of Defined Daily Dose for Animals (DDDvet) and Defined Course Dose for Animals (DCDvet). Available online: https://www.ema.europa.eu/en/documents/scientific-guideline/principles-assignment-defined-daily-dose-animals-dddvet-defined-course-dose-animals-dcdvet_en.pdf, 2015 (accessed on August 31st 2023).*

40. *Indrawan D., Cahyadi E.R., Daryanto A., Hogeveen H. The role of farm business type on biosecurity practices in West Java broiler farms. Prev. Vet. Med. 2020, 176, 104910.*
41. *Chantziaras I., Dewulf J., Van Limbergen T., Klinkenberg M., Palzer A., Pineiro C., Aarstrup Mustsen V., Niemi J., Kyriazakis I., Maes D. Factors associated with specific health, welfare and reproductive performance indicators in pig herds from five EU countries. Preventive veterinary medicine 2018, 159, 106-114.*
42. *Ghent University. Biocheck.UGent. Ghent University – Faculty of Veterinary Medicine – Department of Reproduction, Obstetrics and Herd Health – Veterinary Epidemiology Unit, Merelbeke, Belgium, 2015.*
43. *Laanen M., Persoons D., Ribbens S., De Jong E., Callens B., Strubbe M., Maes D., Dewulf J. Relationship between biosecurity and production/antimicrobial treatment characteristics in pig herds. The Veterinary Journal 2013, 198, 508-512.*
44. *Da Costa M.R., Gasa J., Díaz J.A.C., Postma M., Dewulf J., McCutcheon G., Manzanilla E.G. Using the Biocheck. UGent™ scoring tool in Irish farrow-to-finish pig farms: assessing biosecurity and its relation to productive performance. Porcine health management 2019, 5:4, https://doi.org/10.1186/s40813-018-0113-6.*
45. *Rissala A. Quantitative assessment of biosecurity on Finnish dairy cattle farms by using Biocheck. UGent™ tool (Master's thesis, Eesti Maaülikool), p. 68, 2022.*
46. *Hristov S., Stanković B., Plavšić B., Andrijašević M. Standardi biosigurnosti na farmama goveda. Vodič. Ministarstvo poljoprivrede, šumarstva i vodoprivrede Republike Srbije, Uprava za veterinu (autorizovan materijal), Beograd, Srbija, 2011.*
47. *Hristov S., Stanković B., Plavšić B., Andrijašević M. Standardi biosigurnosti na farmama svinja. Vodič. Ministarstvo poljoprivrede, šumarstva i vodoprivrede Republike Srbije, Uprava za veterinu (autorizovan materijal), Beograd, Srbija, 2011.*
48. *Hristov S., Stanković B., Plavšić B., Andrijašević M. Standardi biosigurnosti na farmama živine. Vodič. Ministarstvo poljoprivrede, šumarstva i vodoprivrede Republike Srbije, Uprava za veterinu (autorizovan materijal), Beograd, Srbija, 2011.*
49. *Hristov S., Stanković B. Welfare and biosecurity indicators evaluation in dairy production. Biotechnology in Animal Husbandry 2009, 25 (5-6), p 623-630.*
50. *Hristov S., Stanković B. Najznačajniji propusti u obezbeđenju dobrobiti životinja na farmama goveda i svinja. Zbornik naučnih radova, Agroekonomik 2009, 3-4, 95-102.*
51. *Hristov S., Plavšić B., Stanković B., Nakov D., Ostojić – Andrić D., Živković V. Consideration of the questionnaire for the assessment of biosecurity measures on pig farms. Poster. COST Action 20103 BETTER - Biosecurity Enhanced Through Training Evaluation and Raising Awareness. Managing Committee Conference in Tirana, Albania, June 20th and 21st, 2023.*
52. *Torremorell M. The Three Levels of Biosecurity of Animals. Professional version. MSD Manual, Veterinary Manual. https://www.msdvetmanual.com/management-and-nutrition/biosecurity/the-three-levels-of-biosecurity-of-animals, 2021 (accessed July 10, 2023).*
53. *Gröndal H., Tuominen K., Sternberg Lewerin S. Perspectives of on-farm biosecurity and disease prevention among selected pig veterinarians and pig farmers in Sweden. Veterinary Record Open 2023, 10(2), e68.*

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Review
Pregledni rad

IMPLEMENTATION OF BIOSECURITY MEASURES IN RUMINANTS FARMS

PRIMENA BIOSIGURNOSNIH MERA NA FARMAMA PREŽIVARA

Branislav Stanković[a]*, SlavčaHristov[a], Dejan Bugarski[b], MilanNinković[c], Branislav Kureljušić[c], Miroslav Kjosevski[d], Ilias Chantziaras[e]

[a]Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade-Zemun, Republic of Serbia
[b]Scientific Veterinary Institute "Novi Sad", Rumenački put 20, 21000 Novi Sad, Republic of Serbia
[c]Institute of Veterinary Medicine of Serbia, Department of Healthcare, Janisa Janulisa 14, Belgrade, Republic of Serbia
[d]Ss. Cyril and Methodius University in Skopje, Faculty of Veterinary Medicine, Lazar Pop-Trajkov 5-7, 1000 Skopje, Republic of North Macedonia
[e]Veterinary Epidemiology Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium;
*Corresponding author: baxton@agrif.bg.ac.rs

B.S. https://orcid.org/0000-0003-3925-6102, S.H. https://orcid.org/0000-0002-1719-9971, D.B. https://orcid.org/0000-0002-6109-1386, M.N. https://orcid.org/0000-0003-0173-7795, B.K. https://orcid.org/0000-0003-4281-1672, M.K. https://orcid.org/0000-0001-5725-4384, I.C. https://orcid.org/0000-0001-5687-9577

ABSTRACT

Biosecurity is the result of all activities designed not only to prevent the introduction and spread of infectious diseases within and between herds, but ultimately to contribute to public health and food safety. Although experts and government agencies recommend the implementation of biosecurity practises on dairy, beef and sheep farms, their use appears to be limited. There is serious lack of training and consequently, rather low level of awareness of biosecurity risks among farmers. They are aware of necessity of good health of animals, but they mostly think of endemic diseases like mastitis, claws disorders, digestive problems, etc.). It seems that they are not aware of contagious disease risks, so pathogens models of spreading and entrance doors in to individual animal or population have to be pointed out. Farmers' opinion is that the government should have a greater input into biosecurity; veterinarians saw the ability or willingness of their clients to invest in biosecurity measures as a major barrier. Therefore, additional evidence of the effectiveness and/or potential economic benefits of proposed on-farm biosecurity measures have be better demonstrated. That would give a solid base for field veterinarians and their organisations to be more proactive in promotion and spreading of information on biosecurity, in both direct contact with farmers and organized training. This important role should be fulfilled in cooperation with farmers associations and veterinary authorities. A legal framework can be helpful, as well as stimulating measures for those who actively and successfully embrace biosecurity concept in their work. In many cases there are problems regarding biosecurity procedures application, particularly in control of movement of humans, animals, vehicles and equipment. Procedures have to be performed much more persistent and in responsible manner, particularly during summer, when field works take a lot of farmers' time. Biosecurity procedures must be more serious and determined as approaching to the object of protection.

Key words: biosecurity, measures, ruminants, farm, implementation

INTRODUCTION

Biosecurity is the result of all activities designed not only to prevent the introduction and spread of infectious diseases within and between herds, but ultimately to contribute to public health and food safety (1). Increasing awareness and attention was initially the result of the livestock industry's efforts to cope with diseases for which vaccines do not exist or have serious limitations (2).

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Although term "biosecurity" refers to management practises to prevent the introduction and spread of infectious diseases (2,3), it has no Oxford English dictionary definition. Interested parties have not only created and adapted different definitions to suit their own particular needs, but it seems that farmers and vets have their own relatively clear definitions of biosecurity in relation to some important diseases that threaten farming, which do not completely match (1).

The mentioned practises related to activities associated with the movement of animals, including the transport vehicles used and the isolation or treatment measures carried out after animals were moved to a holding. Also, biosecurity measures in relation to the sharing of equipment between farms are implemented by producers, as well as all preventive measures implemented by representatives of visiting companies and contractors. In addition, waste disposal and animal access to watercourses (streams, rivers, etc.) need to be taken into account, not to mention practises to reduce the transmission of pathogens within the farm were also analysed, particularly in relation to housing, staff and vehicles on the farm (1).

Although experts and government agencies recommend the implementation of biosecurity practises on dairy farms (4), their use appears to be limited. Rarely used practises include, for example, screening for disease and isolation of new and returning animals, controlling access of visitors or requiring them to wear special footwear and clothing, and using the sick barn exclusively for sick animals (5). As it was mentioned, farmers and vets have their own relatively clear definitions of biosecurity in relation to some important diseases that threaten farming, which do not completely match in the UK. Overall, farmers feel that other stakeholders, such as the government, should have a greater input into biosecurity in Great Britain. Conversely, veterinarians saw the ability or willingness of their clients to invest in biosecurity measures as a major barrier. Veterinarians also felt that additional evidence of the effectiveness and/or potential economic benefits of proposed on-farm biosecurity measures needed to be better demonstrated. The ancillary industries were generally unsure of the role they should play in biosecurity, although study participants highlighted zoonoses as part of the problem and indicated that most of the barriers were at farm level (1).

Even if veterinarians' attitudes towards best on-farm biosecurity practises for large and small ruminants are very sound and scientifically based, there may be some discrepancies and contradictions in their practises. Veterinarians sometimes misjudge their clients' opinion of importance and usefulness of biosecurity. This can lead to mistrust and confusion among farmers and affect their awareness and attitude to act in a timely and correct manner and to follow the proposed biosecurity measures and programmes. Farmers may consider biosecurity measures to be expensive and cumbersome and biosecurity routines at farm level are not always optimal. In some cases (*e.*g. newly introduced diseases), veterinary authorities may also react cautiously or inappropriately due to a temporary lack of reliable information and may be unaware of their ability to improve biosecurity levels on farms through training and sanctions (6,7). Farmers are more likely to act on the information provided by trusted advisors such as vets or by someone with whom they have built up a trusting relationship (8).

ASSESSMENT METHODS

Likewise to creation biosecurity measures, the assessment of their use and efficacy is based on epidemiology knowledge. Central to disease control is the identification of these patterns and the risk factors that increase the likelihood of disease outbreaks, as well as the factors that reduce the likelihood of disease outbreaks, so that measures can be taken to prevent or at least reduce the occurrence, severity and impact of disease (9).

Due to the need to prioritise the surveillance, control and eradication of infectious diseases, many prioritisation or categorisation exercises have been carried out in recent years. Given the lack of prevalence data for most cattle diseases, most of studies have followed the Delphi method (10), which is based on: (a) the establishment of an initial list of diseases, (b) the development of a prioritisation methodology that is translated into a questionnaire, and (c) the ranking or assessment of the different diseases by a panel of experts. This is basically a consensus approach, with many advantages, *e.g.* no need for scientific evidence as it relies on expert opinion, which can be changed through debate, and avoids personal and political influence as consensus is required (11). On the other hand, it should be noted that the opinion and experience of experts cannot replace a confirmatory

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scientific study in all cases, such as multipathogen diseases such as mastitis, respiratory diseases, and diarrhoea, which are usually a major concern for both animal and public health and should not be automatically omitted.

To assess the level of biosecurity on the farm, it is most logical to assess the infection risks for the farm population and its production. For this purpose, Hristov and Stanković (12) developed rather simple and useful questionnaires for cattle, pig and poultry farms. The cattle farm questionnaire is based on 13 indicators (farm biosecurity plan, location and isolation level, introduction of newly acquired animals into the herd, traffic control, attitude towards visitors, feeding and watering control, manure management, attitude towards other animals, rodents and birds' control and sanitation) scoring measure of accomplishment using marks 0-5 (0 – insufficient with no possibility, up to correct, to 5 – excellent), giving average grade for particular farm biosecurity level, expressed from 1 (insufficient, for average score of indicators, 0-1,99) to 5 (excellent, for average score of indicators, 4,50-5). The cattle farm assessment is accompanied with SWOT analysis, which takes into account all remarks when assessing any of individual indicators, suggestions how to improve biosecurity level on the farm.

Nowadays, to assess biosecurity on different types of cattle farms in the most of countries is being used the Biocheck Cattle tool, developed by Damiaans et al. (13). This questionnaire was based on a list of 47 priority diseases for cattle and the risk factors and biosecurity measures associated with these diseases (11), with 19 categories with a total of 304 questions (13).

Lewerin et al. (14) developed the tool to calculate the impact of different biosecurity measures and strategies at the individual farm level. To illustrate the general applicability of the tool, it was applied to theoretical examples of Swedish cattle farms and diseases endemic to these species in the EU in two scenarios with different contact patterns between farms.

BIOSECURITY MEASURES IMPLEMENTATION RESULTS

Biosecurity measures are based on epidemiological principles, and knowing routs of infections and entrance "doors" is crucial for their effectiveness. A key principle of veterinary epidemiology is that diseases do not occur randomly in a population, but in certain members/groups, at certain times and in certain places; diseases follow certain patterns (15). Based on international and national animal health authorities, certain biosecurity measures that contribute to a disease eradication or control programme are mandatory (e.g. winter screening in Belgium to detect potential carriers or vectors and purchased tests for some diseases), while others should be carried out on a voluntary basis (16). Biosecurity practices might differ among and within countries for reasons such as differences in production types, diseases present, legislation on disease control, and available resources (17).

The relationships between direct and indirect causes, factors or attributes and diseases outbreaks are often described as a causal network (18). One of the simplest models of disease causation is the epidemiological triad, the traditional model for infectious diseases (15). This triad consists of an external pathogen, a susceptible host and an environment, including management and husbandry practises that bring the host and pathogen together, where the disease results from the interaction between the pathogen and the susceptible host in an environment that supports the transmission of the pathogen from a source to the host (18).

The mode of transmission, the presence and survival of environmental stages and the presence of reservoirs, carriers and vectors also influence the spread and disease control measures (15). The presence of wildlife can play an important role in the transmission of certain diseases to humans and other animals by serving as vectors for pathogens such as rabies, leptospirosis and paratuberculosis agents (19) or as hosts for parasites such as *Echinococcus spp* (20). Vectors are fundamental for the certain pathogens transmission, such as bluetongue, became more dangerous due to the effects of climate change (21). Finally, the long-term survival of pathogens in the environment is crucial for the spread and persistence and may be used to predict disease emergence (15).

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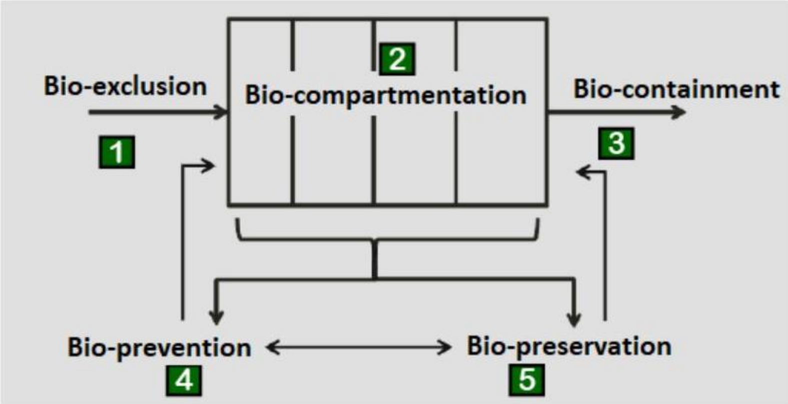

Figure 1. Biosecurity principles and compartments in animal facilities (22)

Biosecurity in animal production can be described through 5 compartments (22), in order to emphasize its importance in protecting animal health, public health and the environment (Figure 1.):

1. Bio-exclusion: biosecurity measures preventing the introduction of a pathogen in a farm,
2. Bio-compartmentation: biosecurity measures preventing the spread of a pathogen within the farm,
3. Bio-containment: biosecurity measures preventing the spread of the pathogen to other farms or premises,
4. Bio-prevention: biosecurity measures preventing the spread of zoonotic pathogens to humans, and
5. Bio-preservation: biosecurity measures preventing environmental contamination(s).

Biosecurity measures are regrouped into different categories and can be related to one or several biosecurity compartments. As an example, applying quarantine for newly purchased animals will contribute to bio-exclusion while a proper carcass disposal system will contribute to bio-compartmentalisation, bio-containment, bio-prevention and bio-preservation (23).

The study of Ferreira et al. (24) described the general and specific biosecurity measures for bovine virus diarrhoea and bovine herpes virus type 1 infection in Campos Gerais region of Paraná, Brazil, and demonstrated their association with the size of dairy farms. The answers, principally those on the access control for people, animals, and vehicles, animal quarantine and isolation, and hygiene practices, varied considerably among the farmers. Biosecurity measures have been poorly adopted in dairy farms. Small dairy farms had the lowest degree of biosecurity. This trend is also observed in dairy, mixed (dairy and beef), and small ruminant farms, which was in accordance with findings of Can and Altuğ (25), who reported the increase in biosecurity with the farm size. Only 15% of farms had visitor policies in place, of which only 4% were small- and medium-size farms. The lack of designated vehicle-parking areas was more common on small farms, and entry of trucks carrying animals for slaughter or disposal was more common on medium-size farms. The practice of purchasing animals was mainly found in medium and large farms (61% and 75%, respectively), which is serious risk factor for small- and medium-size farms for the introduction and spread of BVDV (26). Large farms implement significantly less bioexclusion measures, despite the greater impact of an infectious disease being introduced into the herd (16), so isolation of animals before any contact with domestic herd is necessary, as well diagnostic tests during the quarantine. Additionally, when transporting animals, it is important to avoid mixing batches from different farms and to sanitize the interior and exterior of the transport vehicles. Regarding hygiene practices, sharing of machinery for handling waste and feeding animals was found in most cases, regardless of the size of the production system. Cleaning and disinfection procedures of stalls and vehicles as well as hand washing before handling animals was extensively noticed in medium-size farms, which is essential to prevent the spread of pathogens.

Sahlström et al. (27) found that quarantine was more widely adopted in beef farms than in dairy farms, and was also associated with the size of the production systems. According to Hoe and Ruegg (28), bigger farms isolate sick animals more easily and carry out more diagnostic tests or examinations when new animals are acquired.

Due to the limited alternatives to antimicrobials, biosecurity measures are necessary to ensure their appropriate use. Study of Ferreira et al. (24) found that the most frequently used chemotherapeutics for respiratory diseases in small- and medium-size farms were quinolones and macrolides, whereas large farms preferred amphenicols and macrolides. Large farms tend to use more antimicrobials. Some measures to reduce their use on dairy farms include: cleaning and disinfecting facilities, using replaceable bedding materials, avoiding contact with other farms, and undertaking proper quarantine when introducing new animals, and proper management of mammary gland health (29). Generally, small farms do not have any biosecurity practices in place or avoid certain risk factors, owing to their small size. Medium-size farms are in transition with only a few biosecurity measures. Large farms have the most biosecurity measures, but they face fundamental risk factors owing to the expansion of their farms. When consider the risk score obtained, it is obvious how few biosecurity measures are adopted in general, as the risk levels obtained were high and medium-high.

Biosecurity measures are identified based on the transmission pathways of infectious diseases and risk factors (23). Some of them are disease-specific (*e.g*. vaccination protocols), but the majority of biosecurity measures are generic and contribute to reducing the risks related to several infectious diseases, either by preventing their introduction (*e.g*. applying a quarantine), blocking the transmission pathways (*e.g*. isolation of sick animals) or by reducing the overall infection pressure (*e.g*. general hygiene).

By manipulating the environment, for example by reducing faecal contamination, reducing overcrowding or eliminating carriers or vectors of pathogens, we can reduce disease (20,30), as well as selecting animals resistant to disease or by increasing the resistance of the population through natural or artificial means (15) the severity of the disease may be reduced and thus the impact of the disease. In addition, information about certain host factors, such as age and sex, closely associated with many diseases, is useful for predicting infections within a population or group of animals, and helps to adjust and implement disease control programmes.

Oliveira et al. (31) pointed out insufficient biosecurity adoption in 16 Danish dairy herds, which is risks to animal and human health, besides raising concerns about compromised productivity and animal welfare. Recognized biosecurity practices to prevent introduction and spread of infectious diseases in the herds were broadly discussed by farmers, *e.g*. maintenance of a closed herd status and routines to ensure improved hygiene when handling animals and in the facilities. However, there are farmers expressing relaxed biosecurity attitudes under situations such as the herd's location in a region supposedly less threatened by diseases, need for increased herd size, and values related to keep a farming system without excessive restrictions. Other potential constraints for correct biosecurity adoption included difficult communication between farmers and their employees and visitors, lack of knowledge regarding infection routes, and financial limitations. The farmers received information about biosecurity from several sources, veterinarians being considered the main and trusted one. Beneficial views on the legislation appeared as a way to guarantee mutual adoption of biosecurity among farmers, whereas others believed that legislation might not be needed. Findings of this study showed that, in general, important biosecurity aspects were recognized by the farmers.

Lewerin et al. (14) study model showed that the most important factors influencing the risk and impact of biosecurity measures such as quarantine routines and protective clothing are the frequency of contact between farms and the prevalence of the disease. The risk of introduction and the effect of biosecurity measures varied depending on the type of farm and the transmission route of the disease. The risk assessment model proved useful to illustrate the risk of endemic disease introduction and the mitigating effect of different biosecurity measures at farm level. The results of the model could be used to justify and help veterinary advisors understand farm-specific risks and motivate farmers to improve biosecurity on their farm, as it can be tailored to each farmer’s needs and preferences.

The Biocheck Cattle tool developed by Damiaans et al. (13) is based on risk factors and biosecurity measures related to priority cattle diseases and the results of a cross-sectional survey on Belgian farms with selected questions. The scoring system consists of three separate questionnaires containing 69 (veal), 104 (beef) and 124 (dairy) questions. Experts from different areas of veterinary medicine were asked to weight the different biosecurity categories and questions according to Gore's method. The resulting system provides biosecurity points per category (external

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and internal biosecurity) and sub-category (e.g. purchasing, transport, health management). The Biocheck tool was then used in a survey to assess biosecurity on 20 veal, 50 beef and 50 dairy farms.

Both internal and external biosecurity were categorised as low for all production systems, with low average total biosecurity scores of 39.7 points for veal (SD = 7.4), 44.3 for beef (SD = 8.4) and 48.6 points for dairy producing farms (SD = 8.1), of maximum score of 100 points. The scores for internal biosecurity were lower than for external biosecurity for all farm types. Veal farms scored significantly lower for "purchasing" than beef and dairy farms, while they scored higher for the other subcategories of external biosecurity. For dairy and beef farms, "purchase and reproduction" was the sub-category with the highest score. For internal biosecurity, "health management" was particularly low in the three farm types, while subcategories with more than 50 points were rare. These results prove that this tool can be used to assess the implementation of biosecurity on cattle farms in a standardised and reproducible way, enabling farms benchmarking and herd-specific advising.

The study by Renault et al. (16) reviewed the main findings of various studies conducted from 2015 to 2021 to analyse the biosecurity level on Belgian cattle farms, including the attitudes and behaviours of cattle farmers and farm veterinarians with regard to biosecurity measures. In particular, the aim was to carry out a SWOT analysis (strengths, weaknesses, opportunities and threats) of the situation and to propose a new conceptual framework to improve the level of biosecurity in the cattle sector. Biosecurity in cattle farming is still relatively low and faces numerous challenges. Its future improvement requires that the various stakeholders agree on common objectives and carefully consider animal, human and environmental health as well as socio-economic and cultural factors. Further cost-effectiveness studies are needed to identify the most important biosecurity measures and to convince stakeholders of their benefits and advantages. Cattle farmers rely mainly on rural veterinarians for technical advice and consider them as trusted informants. To promote these good practises more effectively, rural veterinarians need adequate guidance from the authorities, appropriate training in biosecurity and communication, and a favourable environment.

Nevertheless, whilst many of these studies advise the use of preventive procedures, they do not often provide evidence on the efficacies or cost-effectiveness of engaging in such practices (3). The considerable differences in recommendations across publications can lead to confusion among farmers, resulting in them adopting less appropriate practises, which are 'favoured' or easy to implement, but not necessarily the most effective for the farm in question (32).

Biosecurity routines at herd level may reduce the probability of introduction of disease into the herd, but some measures may be regarded as expensive and cumbersome for the farmers (14). Custom-made measures based on individual farm characteristics may aid in improving the actual application of on-farm biosecurity (6,7).

Several aspects, one of which is the perception of the effectiveness of these practises, influence the adoption of preventive behaviour, thus limiting the adoption of biosecurity practises on dairy farms worldwide.

According to Herrmann et al. (33), the implementation of management activities on beef and dairy farms in Germany depend both on the attitude of the people performing the work, and their understanding of why the work should be performed. In the context of animal husbandry, the implementation of such practices is crucial for the functionality of biosecurity. These authors conducted anonymous online survey German farmers and concluded that, in general, farmers are aware of the importance of biosecurity and consequently had concepts of farm biosecurity, which reflected in the current European legislation, since "the operators of farms are responsible for minimizing the risk of the spread of diseases". On the other hand, awareness about introduction routes for animal diseases into a farm was associated with a lack of knowledge of how to improve the measures in these areas. This confirms role of the veterinarian in the context of biosecurity. Overall, the high level of farmers commitment indicated a good implementation of daily practices.

The study of Denis-Robichaud et al. (34) on general understanding of biosecurity and perceptions of the effectiveness of specific biosecurity practises (17 of them) was conducted in 2015 with 368 Canadian dairy farmers. It included correlations between perceptions of the effectiveness of each biosecurity practise and its implementation on the farm. Most respondents felt that the purpose of on-farm biosecurity was to prevent both the introduction of a new pathogen and the spread of an

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existing pathogen (73%) and felt that general biosecurity was effective (92%) and important (58%). Farmers considered most biosecurity measures to be effective (60–94%). Practises related to direct animal-to-animal contact were considered effective by more respondents than biosecurity practises related to contagion and visitors. Less than 20% of them reported having discussed the various biosecurity practises with a veterinarian and less than 60% reported using these practises on their farm. Finally, the reasons given by most farmers for implementing biosecurity measures were that (1) the measure helps to prevent the introduction or spread of a disease regionally and (2) it has been shown to benefit animal health and welfare. These results indicate that the effectiveness of certain biosecurity practises is perceived positively; there may be a lack of understanding of the practises that affect disease transmission through indirect contact, and that the perceived threat of not implementing practises is minimal.

An on-line survey of Renault et al. (11) was implemented in Belgium, France and Spain in order to assess the behaviour of rural veterinarians towards biosecurity, and implementation level of the biosecurity measures. The study identified different strengths, weaknesses, possible constraints and solutions in terms of veterinary perspectives. Veterinarians are considered as key information sources by the farmers, so they should be more active in terms of guidance and improvement of biosecurity at farm level. Two factors seemed to influence significantly the implementation level of measures: the country of practice and the veterinarian’s perception level of biosecurity. The biosecurity stages with the lowest application level, therefore the highest risk were bio-exclusion, increasing the risk of disease introduction, and bio-containment, increasing the risk of inter-herd transmission. Based on this analysis, an initial diseases list needs urgent attention in this part of Europe, since they are all zoonoses: Crimean Congo haemorrhagic fever, Ebola virus disease, Marburg haemorrhagic fever, Lassa fever, Middle East respiratory syndrome (MERS), severe acute respiratory syndrome (SARS), Nipah virus disease and Rift Valley Fever. The study highlighted the areas of improvements.

Biosecurity infrastructures (*e.g.* calving areas, isolation stall) are rarely available in farms. The main weaknesses that should be corrected are linked to bio-exclusion and bio-containment that is footbath and cleaning facilities for visitors, quarantine for newcomers, control of visitors and contacts with other domestic species and wildlife. Regarding the veterinary practices, the current implementation level is rather low, so there is a large place for improvement, for instance, organising the visits on the basis of contamination risks (16).

Using a modified Delphi method, Kuster et al. (17) asked 8 Swiss livestock disease specialists to rank biosecurity measures by allocating a score from 0 (lowest) to 5 (highest) based on their importance related to Swiss legislation, feasibility, as well as the effort required for implementation and the benefit of each biosecurity measure. The biosecurity measures were ranked based on their effectiveness in preventing an infectious agent from entering and spreading on a farm, solely based on transmission characteristics of specific pathogens: bluetongue, bovine viral diarrhoea, foot and mouth disease and infectious bovine rhinotracheitis. For cattle farms, biosecurity measures that improve disease awareness of farmers were ranked as both most important and most effective. Among all single measures evaluated, education of farmers was perceived by the experts to be the most important and effective for protecting Swiss cattle farms from disease. Authors identified the distinction between the terms “importance” and “effectiveness” of biosecurity measures, demanding further research on the effectiveness of biosecurity measures. They call attention to the need for more precise and commonly accepted definitions of biosecurity measures, which would facilitate communication to farmers and policy makers on the value of on-farm biosecurity.

The findings of this study provide an important basis for recommendation to farmers and policy makers. The Swiss approach to maintaining a disease-free livestock population is dominated largely by governmental control measures, with the compulsory bluetongue vaccination in 2008–2010 and the ongoing bovine virus diarrhoea eradication program being notable examples (35,36). In contrast, the implementation of on-farm biosecurity measures in Switzerland is relatively poor. This may be associated with the fact that Swiss livestock herds are still small, despite the global trend towards fewer and bigger enterprises, on average 39 in 2011.

Nöremark and Sternberg-Lewerin (37) investigated professionals visiting animal farms in Sweden perception of the farm biosecurity, the factors that influence their own biosecurity routines and what they describe as obstacles for biosecurity,

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as well as suggestions for improvements. In all groups, a majority of the 386 respondents perceived obstacles for on-farm biosecurity, among veterinarians 66% perceived that there were obstacles, mainly related to the very basics level of biosecurity, such as access to soap and water. Responsibility was identified to be a key issue; while some farmers expect visitors to take responsibility for keeping up biosecurity they do not provide the adequate on-farm conditions. Visitors need to take responsibility for avoiding spread of disease, while farmers need to assume responsibility for providing adequate conditions for on-farm biosecurity.

Toma et al. (38) performed telephone survey on impact of determinants of biosecurity behaviour of 900 cattle and sheep farmers in Great Britain. Their results suggest that farmers' perceived importance of specific biosecurity strategies, organic certification of the farm, knowledge of biosecurity measures, attitudes towards animal welfare, perceived usefulness of biosecurity information sources, perceived impact of severe animal disease outbreaks on the farm in the last five years, membership of a cattle/sheep health programme, attitudes towards biosecurity in animal husbandry, influence on the decision to apply biosecurity measures, experience and economic factors significantly influence behaviour (explaining 64% of the variance in behaviour overall). Numerous studies have shown that farmers make different management decisions at different stages of their lives. Younger farmers with large herds and few dependants are more willing to participate in an eradication programme, whereas older farmers with no offspring are more unwilling to make changes to their management systems (39). Education, experience and cognitive ability are all variables that have a significant impact on the decision making process and are often related to the age of the decision maker. In addition, education and training have been shown to improve and influence farmers' willingness to implement a change in management practises (40). Farmers who experienced disease outbreaks in the past are more likely to use sources of information and apply more biosecurity measures currently on their farms. In addition, farmers who are members in cattle and/or sheep health schemes are likely to apply more biosecurity measures on their farms.

Small-scale family type farms play an important role in the development of the rural economy, reducing poverty among farmers, especially in developing countries. However, this category of producers has faced numerous financial, technical, and legal challenges during last decade (41).

According to Can and Altuğ (25), biosecurity plays a crucial role in preventing contagious diseases and in increasing farm productivity. They conducted on a total of 50 small-scale dairy farms in Hatay, Turkey, in order to determine technical and economic biosecurity scores of farms, and to examine the associations between biosecurity practices and producers' socioeconomic characteristics, using a checklist related to 19 biosecurity practices, using the technical and economic scoring systems of presence and cost of the each of the biosecurity practices. They found that treatment of sick animals (98%), vaccination against the most common contagious diseases (90%), and barn lime (86%) were found to be the most commonly used applications, while testing for the most common contagious diseases before buying (10%) was used at the lowest rate. The authors found significant differences among the groups regarding education level (<.05), income class (<.05), and herd size (<.01). Biosecurity scores were significantly positively correlated with herd size (<.05) and producers' education level (<.01). There were statistically significant associations between the producers' socioeconomic characteristics and some of the biosecurity practices. The authors concluded that training programs should intend to change the attitudes and perception of small-scale producers concerning poor biosecurity practices. In order to encourage producers to increase biosecurity scores, regulations regarding financial support and penalties could be quite useful at both the regional and national levels.

Analysis of biosecurity situation on Serbian dairy, beef and sheep farms of different capacity in graduation theses written by animal science students conducted by Hristov and Stanković during last decade reveals a lot of issues. Biosecurity level is mostly good or very good or seldom sufficient (average grades of farms were in range from 3.85 to 1.49). There is serious lack of training and consequently, rather low level of awareness of biosecurity risks. It indicates that they have to be familiar with pathogens models of spreading and entrance doors in to individual animal or population. Farmers take rather good care of general health of animals (*e.g.* mastitis, claws disorders, digestive problems, etc.), but it seems that they are not aware

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of contagious disease risks. Visitors control is quite liberal, which is general situation in Serbia. Required procedures can be performed much more persistent and in responsible manner, particularly during summer, when field works take a lot of farmers' time. Biosecurity procedures must be more serious and determined as approaching to the object of protection.

CONSTRAINTS AND LIMITATIONS OF BIOSECURITY PROCEDURES IMPLEMENTATION

Historically, the roles and responsibilities of government, industry and animal owners regarding disease control have been based on a set of assumptions that have not always resulted in the major stakeholders working together. Burrell (42) posed the question: "What can producers do to protect themselves regarding disease epidemics?" stating that the industry clearly had a major role to play. One plausible response has been the growth of independent biosecurity initiatives amongst motivated sheep and cattle farmers and between the farmers and their veterinary advisors in several regions of the UK (1).

Toma et al. (38) concluded that the stronger the farmers' attitude towards biosecurity, the more likely they are to apply biosecurity measures on their farms (namely that biosecurity measures are essential for keeping the herd healthy, that the application of biosecurity measures could save farmers a lot of money and that biosecurity regulations are good for animal health). The vast majority of veterinarians consider biosecurity to be a priority for their profession, although they do not consider their own safety to be at risk in their daily practise. This could pose a threat to public health as the seroprevalence for zoonotic diseases tends to be significantly higher among veterinarians in rural areas (43). The survey highlighted weaknesses and areas for improvement, particularly in relation to bio-exclusion (in relation to the risk of disease introduction) and bio-containment (in relation to the risk of disease transmission between herds). In case they do not adopt good practices, veterinarians might be unsuccessful in one of their main responsibilities, to limit the spread of a disease in case of outbreak, and be a high risk for farmers by iatrogenic route of transmission of diseases in premises. Although veterinarians expressed different constraints, possible solutions exist and have already been implemented by some veterinarians, such as an autonomous and mobile decontamination system or farm-dedicated clothes, boots and/or surgical material boxes that are left on premises.

The lack of financial resources can be an important factor for small and medium-size farmers to (not) adopt certain biosecurity measures (44). Simple and cost-effective biosecurity measures can be readily implemented, including communicating access restrictions to visitors, enforcing proper handling of animals, and ensuring that visitors sanitize their hands, boots, and shoes before entering free stalls or bovine facilities. Additionally, having a designated parking area for vehicles is essential and should be emphasized. Cleaning and disinfection procedures are essential to prevent the spread of pathogens, especially it is necessary to clean and disinfect all vehicles that regularly enter a property. Daily cleaning and disinfection of trucks shared for transporting animals are crucial, although these measures may require significant financial investment or employee labour to implement those (24).

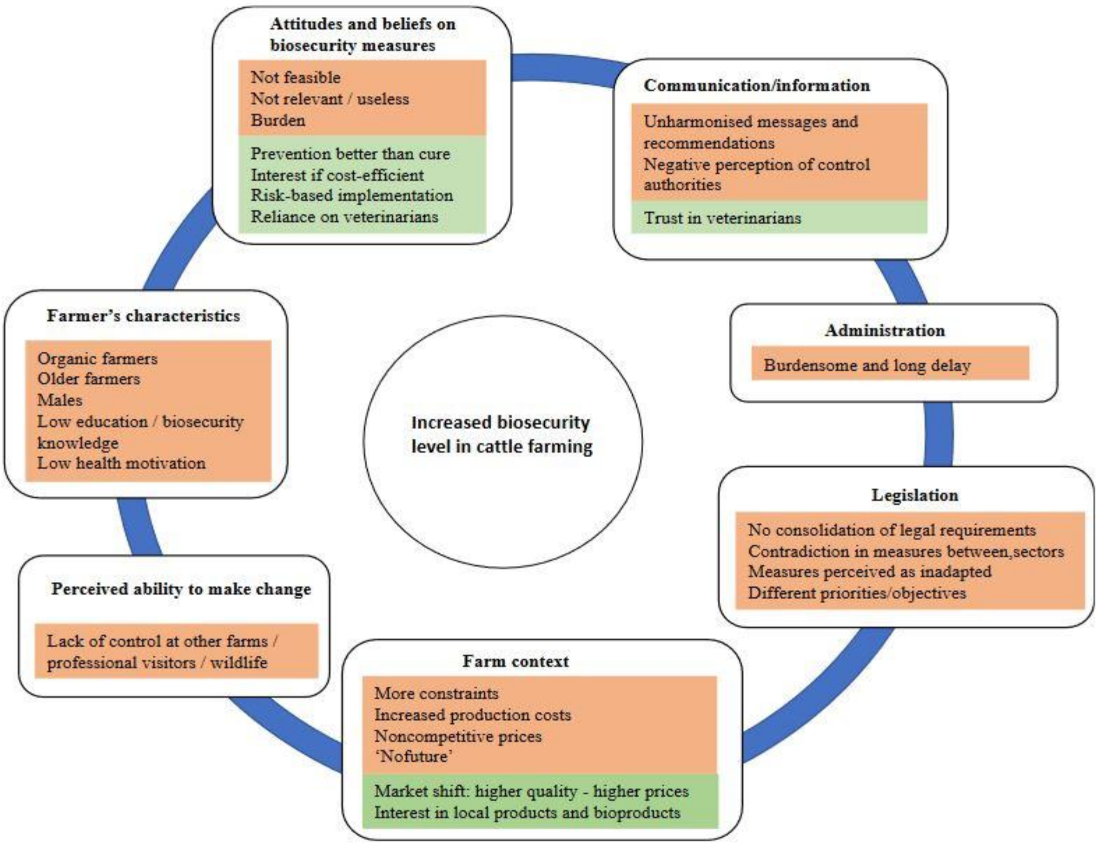

Figure 2. Proposed conceptual model explaining the factors affecting the implementation of biosecurity measures at Belgian cattle farms. In orange, negative-effect factors; in green, positive-effect factors (11)

Renault et al. (11) pointed out that several factors may negatively affect the implementation of biosecurity measures at cattle farms. They are related both to the farmer's attitudes and awareness and the administrative and legal circumstances. Based on this, several recommendations can be made in order to improve the biosecurity level at cattle farms, as it is may be concluded from Figure 2. Authors pointed out that there is an urgent need for evidence-based cost efficiency studies in order to identify the priority biosecurity measures and prove to the farmers of their cost efficiency (28). It was previously mentioned that the veterinarian's perception level of biosecurity measures influences significantly the adequate implementation of good practices. Therefore, it is essential to give biosecurity a greater role in veterinary training programs and curriculum, and to ensure an appropriate and progressive awareness emergent as part of continuing education proposed to veterinarians (11).

The identified measures have to be relevant, adequate, and practicable to the requirements of farmers. They should be shared with other stakeholders in order to stay away from possible contradictions (*e.g.*, removing elements of the "green belt" surrounding farm: bushes and vegetation for vector control, while environmental policy promote natural hedges and prevent vegetation removing). As for the One Health approach, biosecurity measures should be considered as comprehensive, as "a unified concept to integrate human, animal, plant and environmental health" (45). Negative impact of some preventive treatments on the environment or human health has been documented in the past, for instance the development of (multi)drug resistance related to the preventive use of antibiotics in some intensive farming system (46), the contamination of the environment related to treatments of animals with acaricides (47), and the negative effects on beneficial insects consecutive to the use of chemical larvicides in the control of vector breeding sites (48). Negative consequences could be avoided taking the One Health approach, connecting fields of health, agriculture and the environmental protection. Taking into account the natural and social sciences may facilitate the acceptance of biosecurity measures by

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the population (44). These aspects are clearly taken into consideration by the European Green Deal (49), which policy areas include, among other things, biodiversity (measures to protect the ecosystem) and food safety. In addition, effective training focused on chosen goals, and communication to farmers should take place ideally by reputable sources such as veterinary practitioners or farmers' associations, in order to promote biosecurity and the major biosecurity measures, arising stakeholders' knowledge and awareness and reliable legislative document. Last but not least, further studies should obtain convincing evidence of biosecurity measures cost efficiency and recommend the priority measures for their utility and benefits (16). In addition,

Successful implementation of the recommended management practices is related to the farmers risk perception, including acceptable risk and the associated consequences, and the significance of particular biosecurity measure (11). Therefore, the perceived effectiveness of the recommended guidelines, feasibility, and technical knowledge of the subject increase the likelihood of adopting the biosecurity measures in production systems (24). According to the Oliveira et al. (31), insufficient biosecurity adoption in dairy herds has been pointed out. This creates real risks to animal and human health, besides raising concerns about compromised productivity and animal welfare. Nevertheless, in general, farmers are familiar with important biosecurity aspects. Despite of this, factors limiting the adoption of biosecurity measures were present.

CONCLUSIONS

General remark of implementation of biosecurity measures on ruminants' farms worldwide is that it could be done much better. Although is evident that all stakeholders understand they have important role in herd health and production protection against pathogen agents and diseases outbreaks, their knowledge of procedures and measures that have to be undertaken is often very limited. There is serious lack of training and consequently, rather low level of awareness of biosecurity risks. Farmers are aware of necessity of good health of animals, but they mostly think of endemic diseases like mastitis, claws disorders, digestive problems, etc.); it seems that they are not aware of contagious disease risks. It indicates that they have to be familiar with pathogens models of spreading and entrance doors in to individual animal or population. Farmers' opinion is that the government should have a greater input into biosecurity; veterinarians saw the ability or willingness of their clients to invest in biosecurity measures as a major barrier. Additional evidence of the effectiveness and/or potential economic benefits of proposed on-farm biosecurity measures have be better demonstrated. That would give a solid base for field veterinarians and their organisations to be more proactive in promotion and spreading of information on biosecurity, in both direct contact with farmers and organized training. This important role should be fulfilled in cooperation with farmers associations and veterinary authorities. A legal framework can be helpful, as well as stimulating measures for those who actively and successfully embrace biosecurity concept in their work. In many cases there are problems regarding biosecurity procedures application, particularly in control of movement of humans, animals, vehicles and equipment. Procedures have to be be performed much more persistent and in responsible manner, particularly during summer, when field works take a lot of farmers' time. Biosecurity procedures must be more serious and determined as approaching to the object of protection.

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REFERENCES

1. *Gunn G.J., Heffernan C., Hall M., McLeod A., Hovi M. Measuring and comparing constraints to improved biosecurity amongst GB farmers, veterinarians and the auxiliary industries. Preventive veterinary medicine 2008, 84(3-4), 310-323.*
2. *Dargatz D.A., Garry F.B., Traub-Dargatz J.L. An introduction to biosecurity of cattle operations. The Veterinary Clinics of North America. Food Animal Practice 2002, 18(1), 1-5.*
3. *Brennan M.L., Christley R. Biosecurity on cattle farms: A study in North-West England. PLoS ONE 2012, 7, e28139.*

4. *Shortall O., Green M., Brennan M., Wapenaar W., Kaler J. Exploring expert opinion on the practicality and effectiveness of biosecurity measures on dairy farms in the United Kingdom using choice modeling. J. Dairy Sci. 2017, 100:2225–2239.*
5. *Emanuelson, U., Sjöström K., Fall N. Biosecurity and animal disease management in organic and conventional Swedish dairy herds: A questionnaire study. Acta Vet. Scand. 2018, 60:23.*
6. *Kristensen E., Jakobsen E.B. Danish dairy farmers' perception of biosecurity. Prev Vet Med. 2011, 99:122–129.*
7. *Cook A. Implementing biosecurity on dairy farms: Rewriting the 'cultural script'. Vet J. 2013, 197:118–9.*
8. *Blackstock K., Ingram J., Burton R., Brown K., Slee B. Understanding and influencing behaviour change by farmers to improve water quality. Sci. Total Environ. 2010, 408 (23), 5631–5638.*
9. *Rich K.M., Perry B.D. The economic and poverty impacts of animal diseases in developing countries: new roles, new demands for economics and epidemiology. Prev Vet Med 2011, 101(3–4):133–47.*
10. *WHO Setting priorities in communicable disease surveillance. Available at:* http://www.who.int/csr/resources/publications/surveillance/WHO_CDS_EPR_LYO, 2006 *(Accessed 07-05-2024).*
11. *Renault V., Humblet M.F., Moons V., Bosquet G., Gauthier B., Cebrián L.M., Casal J., Saegerman C. Rural veterinarian's perception and practices in terms of biosecurity across three European countries. Transboundary and Emerging Diseases 2018, 65(1), e183-e193.*
12. *Hristov S., Stanković B. Welfare and biosecurity indicators evaluation in dairy production. Biotechnology in Animal Husbandry 2009, 25(5-6): 623-630.*
13. *Damiaans B., Renault V., Sarrazin S., Berge A.C., Pardon B., Saegerman, C., Dewulf J. A risk-based scoring system to quantify biosecurity in cattle production. Preventive veterinary medicine 2020, 179, 104992.*
14. *Lewerin S.S., Österberg J., Alenius S., Elvander M., Fellström C., Tråvén M., Per Wallgren P., Persson Waller K., Jacobson M. Risk assessment as a tool for improving external biosecurity at farm level. BMC Veterinary Research 2015, 11, 1-10.*
15. *Dohoo I.R., Martin W., Stryhn H.E. Veterinary epidemiologic research. 2nd ed., AVC Inc. Charlottetown, 2010.*
16. *Renault V., Humblet M.F., Pham P.N., Saegerman C. Biosecurity at cattle farms: Strengths, weaknesses, opportunities and threats. Pathogens 2021, 10(10), 1315.*
17. *Kuster K., Cousin M.E., Jemmi T., Schüpbach-Regula G., Magouras I. Expert opinion on the perceived effectiveness and importance of on-farm biosecurity measures for cattle and swine farms in Switzerland. PloS one 2015, 10(12), e0144533.*
18. *Martin W. Linking causal concepts, study design, analysis and inference in support of one epidemiology for population health. Prev Vet Med 2008; 86(3.4):270–88.*
19. *Puerto-Parada M., Arango-Sabogal J.C., Paré J., Doré E., Côté G., Wellemans V., Buczinski S., Roy J.P., Labrecque O., Facteau G. Risk factors associated with Mycobacterium avium subsp. paratuberculosis herd status in Québec dairy herds. Prev Vet Med 2018; 152:74–80.*
20. *Abdulhameed M.F., Habib I., Al-Azizz S.A., Robertson. I. Knowledge, awareness and practices regarding cystic echinococcosis among livestock farmers in Basrah Province, Iraq. Vet Sci 2018; 5(1):17–26.*
21. *Thornton P.K., Van de Steeg J., Notenbaert A., Herrero M. The impacts of climate change on livestock and livestock systems in developing countries: a review of what we know and what we need to know. Agric Syst 2009; 101(3):113–27.*
22. *Saegerman C., Dal Pozzo F., Humblet, M.F. Reducing hazards for humans from animals: Emerging and re-emerging zoonoses, Italian Journal of Public Health 2012, 9(2), pp. 13–24.*
23. *Renault V. Biosecurity in Belgian cattle farms: strengths, weaknesses, determining factors and impacts. Universite de Liege, Faculte de medecine veterinaire département des maladies infectieuses et parasitaires service d'epidémiologie et analyse de risques appliquées aux sciences vétérinaires. Doctoral thesis. Pp. 299, 2021.*
24. *Ferreira J.S., Baccili C.C., Nemoto B.S., Vieira F.K., Sviercoski L.M., Ienk T., Tramontini Pagno J., Gomes V. Biosecurity practices in the dairy farms of southern Brazil. Frontiers in Veterinary Science 2024, 11, 1326688.*
25. *Can M.F., Altuğ N. Socioeconomic implications of biosecurity practices in small-scale dairy farms. Veterinary Quarterly 2014, 34(2), 67-73.*
26. *Pinior B., Garcia S., Minviel J.J., Raboisson D. Epidemiological factors and mitigation measures influencing production losses in cattle due to bovine viral diarrhoea virus infection: a meta-analysis. Transbound Emerg Dis. 2019, 66:2426–39.*
27. *Sahlström L., Virtanen T., Kyyrö J., Lyytikäinen T. Biosecurity on Finnish cattle, pig and sheep farms – results from a questionnaire. Prev Vet Med. 2014, 117:59–67.*
28. *Hoe F., Ruegg P. Opinions and practices of Wisconsin dairy producers about biosecurity and animal well-being. J. Dairy Sci. 2006, 89, 2297–2308.*
29. *Dhaka P., Chantziaras, I, Vijay D., Bedi J.S., Makovsk, I., Biebaut E., Dewulf J. Can improved farm biosecurity reduce the need for antimicrobials in food animals? A scoping review. Antibiotics 2023, 12:893.*

ISBN 978-86-7520-611-8

30. Carr J., Howells M. Biosecurity on pig and poultry farms: principles for the veterinary profession. In Pract 2018; 40(6):238–48.
31. Oliveira V.H.S., Anneberg I., Voss H., Sorensen J.T., Thomsen P.T. Attitudes of Danish dairy farmers towards biosecurity. Livest. Sci. 2018, 214: 153–160.
32. Moore D.A., Merryman M.L., Hartman M.L., Klingborg D.J. Comparison of published recommendations regarding biosecurity practices for various production animal species and classes. Journal of the American Veterinary Medical Association 2008, 233: 249–256.
33. Herrmann A., Schulz K., Wischnewski N., Brüssau J., Zeiler E., Sauter-Louis C. Risk Awareness and Attitude of German Farmers towards Biosecurity Measures. Animals 2024, 14(7), 1102.
34. Denis-Robichaud J., Kelton D.F., Bauman C.A., Barkema H.W., Keefe G.P., Dubuc J. Canadian dairy farmers' perception of the efficacy of biosecurity practices. Journal of Dairy Science 2019, 102(11), 10657-10669.
35. Willgert K.J.E., Schroedle B., Schwermer H. Spatial analysis of bluetongue cases and vaccination of Swiss cattle in 2008 and 2009. Geospat Health. 2011; 5: 227–37.
36. Presi P., Struchen R., Knight-Jones T., Scholl S., Heim D. Bovine viral diarrhea (BVD) eradication in Switzerland - Experiences of the first two years. Prev Vet Med. Elsevier B.V. 2011; 99: 112–121.
37. Nöremark M., Sternberg-Lewerin S. On-farm biosecurity as perceived by professionals visiting Swedish farms. Acta Veterinaria Scandinavica 2014, 56, 1-11.
38. Toma L., Stott A.W., Heffernan C., Ringrose S., Gunn G.J. Determinants of biosecurity behaviour of British cattle and sheep farmers—A behavioural economics analysis. Preventive Veterinary Medicine 2013, 108(4), 321-333.
39. Tuyttens F.M., Struelens E., van Gansbeke S., Ampe B., Factors influencing farmers' responses to welfare legislation: a case study of gestation sow housing in Flanders (Belgium). Livestock Sci. 2007, 116, 289–299.
40. Austin E.J., Deary I.J., Willock J. Personality and intelligence as predictors of economic behaviour in Scottish farmers. Eur. J. Personal. 2001, 15, S123–S137.
41. Can MF. A socio-economic analysis of small ruminant breeders' membership relations and organizational effectiveness. Ankara Univ Vet Fak Derg. 2014, 61:119-124.
42. Burrell, A., Animal disease epidemics: implications for production, policy and trade. Outlook Agric. 2002, 31, 151–160.
43. Dal Pozzo F., Martinelle L., Léonard P., Renaville B., Renaville R., Thys C., Smeets F., Czaplicki G., Van Esbroeck M., Saegerman C. Q Fever Serological Survey and Associated Risk Factors in Veterinarians, Southern Belgium, 2013. Transboundary and Emerging Diseases 2017, 64(3), 959–966.
44. Freitas B.B., Correa A.M.F., Valotto A.A., Marcom N.N., Paulino L.R., Brum J.S., Perrota J.H., Barros Filho I.J. Prevalence of bovines persistently infected with bovine viral diarrhea virus (BVDV) in dairy cattle farms in Paraná state, Brazil. Pesquisa Veterinária Brasileira 2021, 41:6622.
45. Hulme P.E. One biosecurity: A unified concept to integrate human, animal, plant, and environmental health. Emerg. Top. Life Sci. 2020, 4, 539–549.
46. Robinson T.P., Wertheim H.F.L., Kakkar M., Kariuki S., Bu D., Price L.B. Animal production and antimicrobial resistance in the clinic. Lancet 2016, 387, e1–e3.
47. De Miranda Santos I.K., Garcia G.R., Oliveira P.S., Verissimo C.J., Katiki L.M., Rodrigues L., Szabó M.P.J., Maritz-Olivier C. Acaricides: Current status and sustainable alternatives for controlling the cattle tick, Rhipicephalus microplus, based on its ecology. In: Ecology and Control of Vector-Borne Diseases; Wageningen Academic Publishers: Wageningen, The Netherlands 2018, 5, 91–134.
48. Milam J.L.F.C.D., Farris J.L., Wilhide J.D. Evaluating mosquito control pesticides for effect on target and nontarget organisms. Arch. Environ. Contam. Toxicol. 2000, 39, 324–328.
49. The European Green Deal. Communication from the Commission to the European Parliament, the European Council, the Council, the European Economic and Social Committee and the Committee of the Regions (COM(2019) 640 final). Policy document. Available at: https://www.eea.europa.eu/policy-documents/com-2019-640-final, 2019 (Accessed 07-05-2024).

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PRIMENA BIOSIGURNOSNIH MERA NA FARMAMA PREŽIVARA (PREVOD)

IMPLEMENTATION OF BIOSECURITY MEASURES IN RUMINANTS FARMS

BranislavStanković [a]*, SlavčaHristov [a], Dejan Bugarski [b], MilanNinković [c], Branislav Kureljušić [c], Miroslav Kjosevski [d], Ilias Chantziaras[e]

[a] Poljoprivredni fakultet Univerziteta u Beogradu, Nemanjina 6, 11080 Beograd-Zemun, Republika Srbija
[b] Naučni institut za veterinarstvo „Novi Sad“, Rumenački put 20, 21000 Novi Sad, Republika Srbija
[c] Institut za veterinu Srbije, Zavod za zdravstvo, Janisa Januliša 14, Beograd, Republika Srbija
[d] Ss. Ćirila i Metodija u Skoplju, Veterinarski fakultet, Lazar Pop-Trajkov 5-7, 1000 Skoplje, Republika Severna Makedonija
[e] Jedinica za veterinarsku epidemiologiju, Odeljenje za reprodukciju, akušerstvo i zdravlje stada, Fakultet veterinarske medicine, Univerzitet Gent, Salisburilaan 133, 9820 Merelbeke, Belgija;
Correspondong author: baxton@agrif.bg.ac.rs

SAŽETAK

Biosigurnost je rezultat svih aktivnosti koje su osmišljene ne samo da spreče unošenje i širenje zaraznih bolesti unutar i između stada, već i da doprinesu javnom zdravlju i bezbednosti hrane. Iako stručnjaci i vladine agencije preporučuju primenu biosigurnosnih praksi na farmama mlečnih i tovnih goveda i ovaca, čini se da je njihova upotreba ograničena. Među odgajivačima postoji ozbiljan nedostatak obuke i, shodno tome, prilično nizak nivo svesti o rizicima biološke bezbednosti. Oni su svesni neophodnosti dobrog zdravlja životinja, ali uglavnom misle o endemičnim bolestima poput mastitisa, poremećaja kandži, problema sa varenjem itd.). Čini se da odgajivači nisu svesni rizika od zaraznih bolesti, pa bi ih trebalo upoznati sa načinima širenja patogenih uzročnika i ulaznim vratima infekcije kod jedinki i populacija. Mišljenje farmera je da bi vlada trebalo da ima veći doprinos u biosigurnost i; veterinari kao glavnu prepreku vide sposobnost ili spremnost svojih klijenata da ulažu u biosigurnosne mere. Zato bi dodatni dokazi o efikasnosti i/ili potencijalnoj koristi od predloženih mera biosigurnosti na farmi trebalo da budu bolje prikazani. To bi obezbedilo solidnu osnovu za terenske veterinare i njihove organizacije da budu proaktivniji u promovisanju i širenju informacija o biosigurnosti, i u direktnom kontaktu sa farmerima, tako i u organizovanju obuke. Ovu važnu ulogu treba ostvariti u saradnji sa udruženjima poljoprivrednika i veterinarskim vlastima. Pravni okvir može biti od pomoći, kao i podsticajne mere za one koji aktivno i uspešno prihvataju koncept biosigurnost i u svom radu. U mnogim slučajevima postoje problemi u vezi sa primenom biosigurnosnim procedurama, posebno u kontroli kretanja ljudi, životinja, vozila i opreme. Postupci se moraju izvoditi mnogo upornije i odgovornije, posebno tokom leta, kada poljski radovi oduzimaju mnogo vremena poljoprivrednicima. Postupci biosigurnosne zaštite moraju biti ozbiljniji i određeni kao približavanje objektu zaštite.

Ključne reči: biosigurnost , mere, preživari, farma, implementacija

UVOD

Biosigurnost predstavlja rezultat svih aktivnosti koje su osmišljene ne samo da spreče unošenje i širenje zaraznih bolesti unutar i između stada, već i da doprinesu javnom zdravlju i bezbednosti hrane (1). Povećanje svesti i pažnje u početku je bio rezultat napora stočara da se izbore sa bolestima za koje vakcine ne postoje ili imaju ozbiljna ograničenja (2).
Iako se pojam „biosigurnost“ odnosi na prakse upravljanja koje imaju za cilj sprečavanja unošenje i širenje zaraznih bolesti (2,3), on nema definiciju Oksfordskog rečnika engleskog jezika. Zainteresovani ne samo da su kreirali i prilagodili različite definicije svojim posebnim potrebama, već se čini da farmeri i veterinari imaju svoje relativno jasne definicije biosigurnosti u odnosu na neke važne bolesti koje prete uzgoju životinja, a koje se ne podudaraju u potpunosti (1) .
Pomenute prakse su se odnosile na aktivnosti vezane za kretanje životinja, uključujući korišćena transportna vozila i mere izolacije ili tretmana koje

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se sprovode nakon preseljenja životinja na imanje. Takođe, mere biosigurnost i u vezi sa deljenjem opreme između farmi sprovode proizvođači, kao i sve preventivne mere koje sprovode predstavnici gostujućih kompanija i izvođača. Pored toga, potrebno je uzeti u obzir odlaganje otpada i pristup životinja vodotocima (potocima, rekama, itd.), a da ne pominjemo i prakse za smanjenje prenosa patogena unutar farme, posebno u vezi sa smeštajem, osobljem i vozila na farmi (1).

Iako stručnjaci i vladine službe preporučuju primenu biosigurnosnih praksi na farmama mleka (4), čini se da je njihova upotreba ograničena. Retko korišćene prakse obuhvataju, na primer, skrining na bolest i izolaciju novih i životinja koje se vraćaju na farmu, kontrolu pristupa posetilaca ili zahtev za njih da nose posebnunu obuću i odeću, uz korišćenje posebnih staja isključivo za bolesne životinje (5). Kao što je pomenuto, farmeri i veterinari imaju svoje relativno jasne definicije biosigurnosti u odnosu na neke važne bolesti koje prete poljoprivredi, a koje se u Velikoj Britaniji ne podudaraju u potpunosti. Sve u svemu, farmeri smatraju da bi druge zainteresovane strane, kao što je vlada, trebalo da imaju veći doprinos biosigurnosti na farmama u Velikoj Britaniji. Nasuprot tome, veterinari su kao glavnu prepreku videli sposobnost ili spremnost svojih klijenata da ulažu u mere biološke bezbednosti. Veterinari su takođe smatrali da je potrebno bolje demonstrirati dodatne dokaze o efikasnosti i/ili potencijalnim ekonomskim koristima predloženih mera biosigurnostina farmi. Pomoćne industrije generalno nisu bile sigurne u ulogu koju bi trebalo da imaju u biosigurnost i, iako su učesnici studije istakli zoonoze kao deo problema i ukazali da je većina barijera na nivou farme (1).

Čak i ako su stavovi veterinara prema najboljim praksama biosigurnosti na farmi za velike i male preživare veoma čvrsti i naučno zasnovani, mogu se pojaviti neslaganja i kontradikcije u njihovim postupcima. Veterinari ponekad pogrešno procenjuju mišljenje svojih klijenata o važnosti i korisnosti biosigurnosti. To može dovesti do nepoverenja i konfuzije među odgajivačima i uticati na njihovu svest i stav da deluju blagovremeno i ispravno i prate predložene mere i programe biosigurnosti.

Odgajivači mogu smatrati da su mere biosigurnostiskupe i glomazne, a i rutininske biosigurnosne mere na nivou farme nisu uvek optimalne. U nekim slučajevima (npr. bolesti koje se od skora pojavljuju), veterinarske vlasti takođe mogu reagovati oprezno ili neodgovarajuće zbog privremenog nedostatka pouzdanih informacija i mogu biti nesvesne svoje sposobnosti da poboljšaju nivoe biosigurnosti na farmama kroz obuku i sankcije (6,7). Veća je verovatnoća da će farmeri delovati na osnovu informacija koje im daju pouzdani savetnici kao što su veterinari ili neko sa kim su izgradili odnos poverenja (8).

METODE PROCENE

Kao i kod osmišljavanja biosigurnosnih mera, procena njihove upotrebe i efikasnosti zasniva se na poznavanju epidemiologije. Ključno u kontroli bolesti je prepoznavanje obrazaca širenja bolesti i faktora rizika koji povećavaju verovatnoću izbijanja, kao i faktora koji smanjuju verovatnoću izbijanja bolesti, tako da se mogu preduzeti mere za sprečavanje ili bar smanjenje pojave, ozbiljnost i uticaj bolesti (9).

Zbog potrebe da se da prioritet nadzoru, kontroli i iskorenjivanju zaraznih bolesti, poslednjih godina su sprovedene mnoge vežbe sagledavanja prioriteta ili kategorizacije. S obzirom na nedostatak podataka o prevalenci za većinu bolesti goveda, većina studija je pratila Delphi metod (10), koji se zasniva na: (a) uspostavljanju početne liste bolesti, (b) razvoju metodologije prioritizacije koja se prevodi u upitnik, i (c) rangiranje ili procena različitih bolesti od strane panela stručnjaka.

Ovo je u osnovi pristup konsenzusom, sa mnogo prednosti, gde na primer nema potrebe za naučnim dokazima jer se oslanja na stručno mišljenje, koje se može promeniti kroz debatu, i izbegava lični i politički uticaj pošto je potreban konsenzus (11). S druge strane, treba napomenuti da mišljenje i iskustvo stručnjaka ne mogu zameniti potvrdnu naučnu studiju u svim slučajevima, kao što su multipatogene bolesti kao što su mastitis, respiratorna oboljenja i dijareja, koji obično predstavljaju veliku zabrinutost i za životinje i za životinje. javno zdravlje i ne bi trebalo automatski da se izostavi.

Da bi se procenio nivo biosigurnosti na farmi, najlogičnije je proceniti rizike od infekcije za populaciju farme i njenu proizvodnju. U tu svrhu Hristov i Stanković (12) su razvili prilično jednostavne i korisne upitnike za farme goveda, svinja i živine. Upitnik farme goveda je zasnovan na 13 indikatora (plan biosigurnost i farme, lokacija i nivo izolacije, uvođenje novonabavljenih životinja u stado, kontrola saobraćaja, odnos prema posetiocima, kontrola hranjenja i pojenja,

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upravljanje stajnjakom, odnos prema drugim životinjama, glodarima i kontrola ptica i sanitacija) bodovanje mera postignuća koristeći ocene 0-5 (0 – nedovoljno bez mogućnosti, do tačne, do 5 – odlično), dajući prosečnu ocenu za određeni nivo biosigurnosti farme, izraženu sa 1 (nedovoljno, za prosek ocena indikatora, 0-1,99) do 5 (odličan, za prosečnu ocenu indikatora 4,50-5). Procena farme goveda je praćena SVOT analizom, koja uzima u obzir sve primedbe prilikom procene bilo kog pojedinačnog indikatora, sugestije kako da se poboljša nivo biosigurnost i na farmi.

Danas se za procenu biosigurnosti na različitim tipovima farmi goveda u većini zemalja koristi Biocheck Cattle alat, koji su razvili Damiaans i sar. (13). Ovaj upitnik je zasnovan na listi od 47 prioritetnih bolesti za goveda i faktora rizika i biobezbednosnih mera povezanih sa ovim bolestima (11), sa 19 kategorija sa ukupno 304 pitanja (13).

Leverin i sar. (14) su razvili alat za izračunavanje uticaja različitih mera i strategija biosigurnosti na nivou individualne farme. Da bi se ilustrovala opšta primenljivost alata, primenjen je na teorijske primere švedskih farmi goveda i bolesti endemskih za ove vrste u EU u dva scenarija sa različitim obrascima kontakta između farmi.

REZULTATI PRIMENE MERA BIOSIGURNOSTI

Mere biosigurnosti su zasnovane na epidemiološkim principima, a poznavanje puteva infekcija i ulaznih „vrata“ je ključno za njihovu efikasnost. Ključni princip veterinarske epidemiologije je da se bolesti ne javljaju nasumično u populaciji, već u jedinkama/grupama, u određeno vreme i na određenim mestima; bolesti prate određene obrasce (15).

Prema međunarodnim i nacionalnim autoritetima za zdravlje životinja, određene mere biosigurnosti koje doprinose programu iskorenjivanja ili kontrole bolesti su obavezne (npr. zimski skrining u Belgiji radi otkrivanja potencijalnih nosilaca ili vektora i kupljeni testovi za neke bolesti), dok druge treba sprovesti na na dobrovoljnoj osnovi (16). Praksa biosigurnosti se može razlikovati među zemljama i unutar njih usled razlika u tipovima proizvodnje, prisutnim bolestima, zakonodavstvu o kontroli bolesti i dostupnim resursima (17).

Odnosi između direktnih i indirektnih uzroka, faktora ili atributa i izbijanja bolesti često se opisuju kao uzročna mreža (18). Jedan od najjednostavnijih modela uzročnosti bolesti je epidemiološka trijada, tradicionalni model zaraznih bolesti (15). Ova trijada se sastoji od spoljašnjeg patogena, osetljivog domaćina i okruženja, uključujući postupke upravljanja i uzgoja koji spajaju domaćina i patogena, gde je bolest rezultat interakcije između patogena i osetljivog domaćina u okruženju koje podržava prenošenje patogen od izvora do domaćina (18).

Način prenošenja, prisustvo i opstanak razvojnih oblika i prisustvo rezervoara, nosilaca i vektora takođe utiču na širenje i mere kontrole bolesti (15). Prisustvo divljih životinja može igrati važnu ulogu u prenošenju određenih bolesti na ljude i druge životinje služeći kao vektori za patogene kao što su besnilo, leptospiroza i uzročnici paratuberkuloze (19) ili kao domaćini za parazite kao što je Echinococcus spp (20). Vektori su od suštinskog značaja za prenošenje određenih patogena, kao što je bolest plavog jezika, koja je postala opasnija zbog efekata klimatskih promena (21). Konačno, dugoročni opstanak patogena u životnoj sredini je ključan za širenje i perzistentnost i može se koristiti za predviđanje pojave bolesti (15).

Slika 1. Principi biosigurnosti i odeljenja u objektima za životinje (22) – pogledati englesku verziju rada

Biosigurnost u stočarskoj proizvodnji može se opisati kroz 5 elemenata (22), kako bi se istakao njen značaj u zaštiti zdravlja životinja, javnog zdravlja i životne sredine (Slika 1.):

1. Bio-isključenje: mere biosigurnosti koje sprečavaju unošenje patogena na farmu,
2. Bio-kompartmentacija: mere biosigurnosti koje sprečavaju širenje patogena unutar farme,
3. Bio-zadržavanje: mere biosigurnosti koje sprečavaju širenje patogena na druge farme ili prostorije,
4. Bio-prevencija: mere biosigurnosti koje sprečavaju širenje zoonotskih patogena na ljude, i
5. Bio-očuvanje: mere biosigurnosti koje sprečavaju kontaminaciju(e) životne sredine.

Mere biosigurnosti su pregrupisane u različite kategorije i mogu se odnositi na jedan ili više odeljaka biosigurnost i. Na primer, primena karantina za novokupljene životinje će doprineti bio-isključenju, dok će pravilan sistem odlaganja leševa doprineti bio-kompartmentalizaciji, biološkom zadržavanju, bio-prevenciji i bio-očuvanju (23).

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Studija Ferreira i sar. (24) je prikazala opšte i specifične mere biosigurnosti za bovinu virusnu dijareju i infekciju virusom goveđeg herpesa tipa 1 u regionu Campos Gerais u Parani u Brazilu, i pokazala njihovu povezanost sa kapacitetom farmi muznih krava. Odgovori u vezi kontrole pristupa za ljude, životinje i vozila, karantinu i izolaciji životinja i higijenskih praksi su se značajno razlikovali među farmerima. Mere biosigurnost i su loše usvojene na farmama muznih krava. Najniži stepen biosigurnosti imale su farme malog kapaciteta. Ovaj trend je primećen i na farmama muznih krava, farmama mešovitog tipa (mleko i meso) i farmama malih preživara, što je u skladu sa nalazima Cana i Altuga (25), koji su ustanovili povećanje nivoa biosigurnosti sa povećanjem veličine farme. Samo 15% farmi je imalo uspostavljen režim za posetioce, od kojih su samo 4% bila mala i srednja gazdinstva.

Nedostatak parkinga za vozila je bio češći na malim farmama, a ulazak kamiona koji su prevozili životinje na klanje ili odlaganje bio je češći na farmama srednje veličine. Praksa nabavke i uvođenja životinja uglavnom je uočena na srednjim i velikim farmama (61% i 75%, respektivno), što je ozbiljan faktor rizika za mala i srednja gazdinstva u pogledu unošenja i širenja BVDV (26). Velike farme sprovode znatno manje mera bioisključivanja, uprkos većem uticaju zarazne bolesti koja se unosi u stado (16), pa je neophodna izolacija životinja pre bilo kakvog kontakta sa domaćim stadom, kao i dijagnostički testovi tokom karantina. Pored toga, prilikom transporta životinja je važno izbegavati mešanje grupa poreklom sa različitih farmi i dezinfikovati unutrašnjost i spoljašnjost transportnih vozila. Što se tiče higijenske prakse, u većini slučajeva je konstatovano deljenje mašina za rukovanje otpadom i ishranu životinja između gazdinstava, bez obzira na veličinu proizvodnog sistema. Postupci čišćenja i dezinfekcije staja i vozila, kao i pranje ruku pre rukovanja životinjama, su uobičajeni na farmama srednje veličine, što je neophodno za sprečavanje širenja patogena.

Sahlström i sar. (27) su ustanovili da je karantin češće usvojena mera na farmama tovnih goveda nego na farmama muznih krava, a takođe je povezan sa veličinom proizvodnih sistema. Prema Hoe and Ruegg-u (28), veće farme lakše izoluju bolesne životinje i sprovode više dijagnostičkih testova ili pregleda kada se nabave nove životinje.

Zbog ograničenih alternativa antimikrobnim sredstvima, neophodne su mere biosigurnosti kako bi se obezbedila njihova odgovarajuća upotreba. Studija Ferreira i sar. (24) su otkrili da su najčešće korišćeni hemoterapeutici za respiratorne bolesti na malim i srednjim farmama hinoloni i makrolidi, dok na velikim farmama preovlađuju amfenikoli i makrolidi, uz primenu više različitih antimikrobnih sredstava. Mere za smanjenje njihove upotrebe na farmama mleka uključuju čišćenje i dezinfekciju objekata, korišćenje zamenljivog materijala za posteljinu, izbegavanje kontakta sa drugim farmama i preduzimanje odgovarajućeg karantina prilikom uvođenja novih životinja i pravilno upravljanje zdravljem mlečne žlezde (29). Uopšteno govoreći, male farme nemaju uspostavljene prakse biosigurnosti ili izbegavaju određene faktore rizika, zbog svoje male veličine. Farme srednje veličine su u tranziciji sa samo nekoliko mera biološke bezbednosti. Velike farme imaju najviše mera biološke bezbednosti, ali se suočavaju sa elementarnim faktorima rizika zbog širenja svojih farmi. Kada se uzme u obzir dobijena ocena rizika, očigledno je koliko je malo mera biosigurnosti uopšte usvojeno, pošto su dobijeni nivoi rizika bili visoki i srednje visoki.

Mere biosigurnosti se identifikuju na osnovu puteva prenošenja zaraznih bolesti i faktora rizika (23). Neke od njih su specifične za bolest (npr. protokoli vakcinacije), ali većina mera biosigurnostije generička i doprinosi smanjenju rizika povezanih sa nekoliko zaraznih bolesti, bilo sprečavanjem njihovog unošenja (npr. primenom karantina), blokiranjem puteva prenosa (npr. izolacijom bolesnih životinja) ili smanjenjem ukupnog pritiska infekcije (npr. podizanjem nivoa opšte higijene).

Pravilnim postupanjem u okolini, na primer smanjenjem fekalne kontaminacije, smanjenjem prenaseljenosti ili eliminacijom nosilaca ili vektora patogena je moguće smanjiti verovatnoću pojave bolesti (20,30). To je moguće postići i selekcijom životinja otpornih na bolesti ili povećanjem otpornosti populacije kroz prirodne ili veštačkim sredstvima (15), a samim tim i uticaj bolesti. Pored toga, informacije o određenim faktorima domaćina, kao što su starost i pol, usko povezani sa mnogim bolestima, korisne su za predviđanje infekcija unutar populacije ili grupe životinja i pomažu u prilagođavanju i primeni programa kontrole bolesti.

Oliveira i sar (31) su ukazali na nedovoljno usvajanje biosigurnosti u 16 danskih mlečnih stada, što predstavlja rizik po zdravlje životinja i ljudi, pored ugrožene produktivnosti i dobrobiti životinja. Odgajivačma su poznate priznate biosigurnosti prakse za sprečavanje unošenja i širenja zaraznih

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bolesti u stadima, npr. održavanje statusa zatvorenog stada i rutine kako bi se obezbedila poboljšana higijena pri rukovanju životinjama iu objektima. Međutim, postoje odgajivači koji izražavaju opuštene stavove o biosigurnosti u situacijama kao što su lokacija stada u regionu koji je navodno manje ugrožen od bolesti, potreba za povećanjem veličine stada i vrednosti koje se odnose na održavanje sistema poljoprivrede bez preteranih ograničenja. Ostala moguća ograničenja za pravilno usvajanje biosigurnosti uključivala su otežanu komunikaciju između farmera i njihovih zaposlenih i posetilaca, nedostatak znanja o putevima širenja infekcije i finansijska ograničenja. Poljoprivrednici su informacije o biosigurnosti dobijali iz više izvora, a veterinari se smatraju glavnim i pouzdanim. Pozitivni stavovi o zakonodavstvu su se pojavili kao način da se garantuje uzajamno usvajanje biosigurnosti među poljoprivrednicima, dok su drugi verovali da zakon možda neće biti potreban. Nalazi ove studije su pokazali da su, generalno, poljoprivrednici prepoznali važne aspekte biološke bezbednosti.

Studija model Leverin i sar. (14) je pokazao da su najvažniji faktori koji utiču na rizik i uticaj biosigurnosnih mera kao što su karantin i nošenje zaštitne, odeće učestalost kontakta između farmi i prevalencija bolesti. Rizik od unošenja i efekat biosigurnosnih mera varirao je u zavisnosti od vrste farme i puta prenosa bolesti. Model procene rizika se pokazao korisnim za ilustraciju rizika od unošenja endemske bolesti i ublažavajući efekat različitih mera biosigurnosti na nivou farme. Rezultati modela bi se mogli koristiti da opravdaju i pomognu veterinarskim savetnicima da razumeju rizike specifične za farmu i motivišu farmere da poboljšaju biosigurnost na svojoj farmi, jer se može prilagoditi potrebama i preferencijama svakog farmera.

Biocheck Cattle alat koji je razvio Damiaans i sar. (13) se zasniva na faktorima rizika i merama biosigurnosti u vezi sa prioritetnim bolestima goveda i rezultatima ankete preseka na belgijskim farmama sa odabranim pitanjima. Sistem bodovanja se sastoji od tri odvojena upitnika koji sadrže 69 (tov teladi), 104 (tov junadi) i 124 (proizvodnja mleka) pitanja. Stručnjaci iz različitih oblasti veterinarske medicine zamoljeni su da odmere različite kategorije biosigurnosti i pitanja prema Goreovom metodu. Dobijeni sistem obezbeđuje bodove biosigurnosti po kategoriji (spoljna i unutrašnja biosigurnost) i podkategorijama (npr. nabavka, transport, zdravstveni menadžment). Biocheck alat je zatim korišćen u istraživanju za procenu nivoa biosigurnosti na 20 farmi za tov teladi, 50 farmi za tov junadi i 50 farmi za proizvodnju mleka.I unutrašnja i eksterna biosigurnost su kategorisane kao niske za sve proizvodne sisteme, sa niskim prosečnim ukupnim ocenama biosigurnosti od 39,7 poena za tov teladi (SD = 7,4), 44,3 za tov junadi (SD = 8,4) i 48,6 poena za farme muznih krava (SD = 8,1) od maksimalnih 100 poena. Rezultati za unutrašnju biosigurnost bili su niži nego za eksternu biosigurnost za sve tipove farmi. Farme za tov junadi su imale znatno niže rezultate za uvođenjenovih grla od farmi tovnih goveda i farmi muznih krava, dok su za ostale potkategorije eksterne biosigurnost i dobile veći rezultat. Za farme muznih krava i tov goveda nabavka i reprodukcija su najviše ocenjene. Za internu biosigurnost, upravljanje zdravljem je bilo posebno nisko u tri tipa farmi, farme sa više od 50 poena bile retke. Ovi rezultati dokazuju da se ovaj alat može koristiti za procenu primene biosigurnost i na farmama goveda na standardizovan i ponovljiv način. Ovo će omogućiti kategorizaciju farmi i kvalitetno savetovanje za svako stado ponaosob.

Studija Renault i sar. (16) je prikazala glavne nalaze različitih studija sprovedenih od 2015. do 2021. godine kako bi se analizirao nivo biosigurnosti na belgijskim farmama goveda, uključujući stavove i ponašanja stočara i veterinara farmi u vezi sa merama biološke bezbednosti. Konkretno, cilj je bio da se izvrši SWOT analiza (snage, slabosti, prilike i pretnje) situacije i da se predloži novi konceptualni okvir za poboljšanje nivoa biosigurnosti u sektoru stočarstva. Nivo primene biosigurnosnih u stočarstvu je još uvek relativno nizak i suočava se sa brojnim izazovima.

Podizanje nivoa biosigurnosti stočarske proizvodnje zahteva da se različite zainteresovane strane dogovore oko zajedničkih ciljeva i pažljivo razmotre zdravlje životinja, ljudi i životne sredine, kao i društveno-ekonomske i kulturne faktore. Potrebne su dalje studije isplativosti da bi se identifikovale najvažnije mere biološke bezbednosti i da bi se zainteresovane strane uverile u njihove prednosti i prednosti. Stočari se uglavnom oslanjaju na veterinare sa kojma sarađuju za tehničke savete i smatraju ih pouzdanim izvorima informacija. Da bi efikasnije promovisali ove dobre prakse, ruralnim veterinarima su potrebne adekvatne smernice od strane vlasti, odgovarajuća obuka o biološkoj bezbednosti i komunikaciji i povoljno okruženje. Ipak, iako mnoge od ovih studija savetuju upotrebu

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preventivnih procedura, one često ne pružaju dokaze o efikasnosti ili isplativosti angažovanja u takvim praksama (3). Značajne razlike u preporukama u publikacijama mogu dovesti do zabune među poljoprivrednicima, što rezultira usvajanjem manje odgovarajućih praksi, koje su „poželjne" ili jednostavne za implementaciju, ali ne nužno i najefikasnije za farmu o kojoj je reč (32). Rutinski postupci biosigurnosnih mera na nivou stada mogu smanjiti verovatnoću unošenja bolesti u stado, ali se neke mere mogu smatrati skupim i glomaznim za farmere (14). Mere napravljene po meri zasnovane na individualnim karakteristikama farme mogu pomoći u poboljšanju stvarne primene biobezbednosti na farmi (6,7). Više aspekata, od kojih je jedan percepcija efikasnosti ovih praksi, utiče na usvajanje preventivnog ponašanja, čime se ograničava usvajanje praksi biosigurnosti na farmama mleka širom sveta.

Prema Herrmannu i sar. (33), sprovođenje aktivnosti upravljanja na farmama za tov i proizvodnju mleka u Nemačkoj zavisi kako od stava ljudi koji obavljaju posao, tako i od njihovog razumevanja zašto posao treba da se obavlja. U kontekstu stočarstva, primena takvih praksi je ključna za funkcionalnost biosigurnosti. Ovi autori su sproveli anonimnu onlajn anketu nemačkih farmera i zaključili da su, generalno, farmeri svesni značaja biosigurnosti i da su shodno tome imali koncepte biosigurnosti farme, koji se odražavaju u sadašnjem evropskom zakonodavstvu, budući da su „operateri farmi odgovorni za minimiziranje rizik od širenja bolesti". S druge strane, svest o putevima unošenja bolesti životinja na farmu bila je povezana sa nedostatkom znanja o tome kako unaprediti mere u ovim oblastima. Ovo potvrđuje ulogu veterinara u kontekstu biosigurnosti. Sve u svemu, visok nivo posvećenosti farmera ukazuje na dobru primenu svakodnevnih praksi.

Studija Denis-Robichaud i sar. (34) o opštem razumevanju biosigurnosti i percepciji delotvornosti specifičnih praksi biosigurnosti (njih 17) sprovedeno je 2015. sa 368 kanadskih farmera. To je uključivalo korelacije između percepcije efikasnosti svake prakse biosigurnosti i njene primene na farmi. Većina ispitanika smatra da je svrha biosigurnosti na farmi da spreči i uvođenje novog patogena i širenje postojećeg patogena (73%) i smatra da je opšta biološka bezbednost efikasna (92%) i važna (58%). Poljoprivrednici smatraju da je većina mera biološke bezbednosti delotvorna (60–94%). Više ispitanika je smatralo da su prakse koje se odnose na direktan kontakt životinja-životinja efikasnije nego prakse biološke bezbednosti koje se odnose na zarazu i posetioce. Manje od 20% njih izjavilo je da su razgovarali o različitim praksama biosigurnosti sa veterinarom, a manje od 60% je izjavilo da koristi ove prakse na svojoj farmi. Konačno, razlozi koje je većina farmera navela za primenu mera biosigurnosti bili su da (1) ta mera pomaže u sprečavanju unošenja ili širenja bolesti na regionalnom nivou i (2) da se pokazalo da koristi zdravlju i dobrobiti životinja. Ovi rezultati ukazuju na to da se efikasnost određenih biobezbednosnih praksi doživljava pozitivno; može postojati nedostatak razumevanja praksi koje utiču na prenošenje bolesti putem indirektnog kontakta i da je uočena pretnja nesprovođenja praksi minimalna.

On-line istraživanje Renault i sar. (11) je sprovedeno u Belgiji, Francuskoj i Španiji kako bi se procenilo ponašanje seoskih veterinara prema biološkoj bezbednosti i nivo implementacije mera biobezbednosti. Studija je identifikovala različite prednosti, slabosti, moguća ograničenja i rešenja u pogledu veterinarskih perspektiva. Veterinari se smatraju ključnim izvorima informacija od strane farmera, tako da bi trebali biti aktivniji u smislu vođenja i poboljšanja biosigurnosti na nivou farme. Činilo se da su dva faktora značajno uticala na nivo implementacije mera: zemlja prakse i nivo percepcije veterinara o biološkoj bezbednosti. Faze biosigurnosti sa najnižim nivoom primene, samim tim i najvećim rizikom bile su bio-isključenje, povećavajući rizik od unošenja bolesti, i bio-sadržaj, povećavajući rizik od transmisije među stadom. Na osnovu ove analize, početna lista bolesti zahteva hitnu pažnju u ovom delu Evrope, jer su sve zoonoze: hemoragična groznica Krimskog Konga, bolest virusa ebole, Marburg hemoragična groznica, Lasa groznica, bliskoistočni respiratorni sindrom (MERS), teški akutni respiratorni sindrom (SARS), bolest virusa Nipah i groznica doline Rift.

Infrastruktura biosigurnosti (npr. područja za teljenje, izolaciona staja) retko je dostupna na farmama. Glavne slabosti koje bi trebalo ispraviti su vezane za bio-isključenje i bio-zadržavanje, a to su tuševi i prostorije za sanitaciju za posetioce, karantin za novonabavljena grla, kontrola posetilaca i kontakti sa drugim domaćim vrstama i divljim životinjama. Što se tiče veterinarske prakse, trenutni nivo implementacije je prilično nizak, tako da postoji veliki prostor za poboljšanje, na primer, organizovanje poseta na osnovu rizika od kontaminacije (16).

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Koristeći modifikovanu Delphi metodu, Kuster i sar. (17) su potražili od 8 švajcarskih veterinarskih specijalista da rangiraju mere biosigurnosti tako što će dodeliti ocenu od 0 (najniži) do 5 (najviši) na osnovu njihovog značaja u vezi sa švajcarskim zakonodavstvom, izvodljivosti, kao i napora potrebnih za implementaciju i koristi od svaka mera biološke bezbednosti. Biosigurnosne mere su rangirane na osnovu njihove efikasnosti u sprečavanju ulaska infektivnog agensa i širenja na farmi, isključivo na osnovu karakteristika prenosa specifičnih patogena: bolesti plavog jezika, goveđe virusne dijareje, slinavke i šapa i infektivnog goveđeg rinotraheitisa. Za farme goveda, biosigurnosne mere koje podižu svest farmera o bolestima su rangirane kao najvažnije i najefikasnije. Među svim evaluiranim pojedinačnim merama, edukacija farmera stručnjaci su ocenili kao najvažniju i najefikasniju za zaštitu švajcarskih govedarskih farmi od bolesti. Autori su identifikovali razliku između pojmova „važnost" i „efikasnost" biobezbednosnih mera, zahtevajući dalja istraživanja o efikasnosti mera biobezbednosti. Oni skreću pažnju na potrebu za preciznijim i opšteprihvaćenim definicijama biosigurnosnih mera, koje bi olakšale komunikaciju sa farmerima i kreatorima politike o značaju biosigurnosti na farmi. Nalazi ove studije predstavljaju važnu osnovu za preporuku poljoprivrednicima i kreatorima politike. Švajcarskim pristupom održavanju populacije stoke bez bolesti uglavnom dominiraju vladine mere kontrole, uz obaveznu vakcinaciju protiv bolesti plavog jezika 2008–2010. i tekući program iskorenjivanja dijareje goveđeg virusa (35,36). Nasuprot tome, primena biobezbednosnih mera na farmi u Švajcarskoj je relativno loša, što se može povezati sa činjenicom da su švajcarska stada, uprkos globalnom trendu povećanja, i dalje relativno mala.

Nöremark i Sternberg-Leverin (37) su istraživali percepciju profesionalaca koji posećuju farme u Švedskoj o farmskoj biosigurnosti, faktorima koji utiču na njihove biosigurnosne rutine i šta oni opisuju kao prepreke za biobezbednost, kao i predloge za poboljšanje. U svim grupama, većina od 386 ispitanika uočila je prepreke za biosigurnost na farmi, među veterinarima 66% je smatralo da postoje prepreke, uglavnom vezane za sam osnovni nivo biosigurnosti, kao što je pristup sapunu i vodi. Odgovornost je identifikovana kao ključno pitanje; dok neki farmeri očekuju da posetioci preuzmu odgovornost za održavanje biosigurnost, oni ne obezbeđuju adekvatne uslove na farmi. Posetioci treba da preuzmu odgovornost za izbegavanje širenja bolesti, dok farmeri treba da preuzmu odgovornost za obezbeđivanje adekvatnih uslova za biosigurnost na farmi.

Toma i sar. (38) su uradili telefonsku anketu o uticaju determinanti biobezbednosnog ponašanja 900 farmera goveda i ovaca u Velikoj Britaniji i ustanovili da farmeri prihvataju značaj specifičnih strategija biosigurnosti, organske sertifikacije farme, poznavanje mera biološke bezbednosti, stavova prema dobrobiti životinja, uočenu korist od izvora informacija o biološkoj bezbednosti, uočeni uticaj izbijanja teških bolesti životinja na farmama u poslednjih pet godina, članstvo u programima zaštite zdravlja goveda/ovaca, stavovi prema biosigurnosti u stočarstvu, uticaj na odluku o primeni biosigurnosnih mera, iskustvo i ekonomski faktori značajno utiču na ponašanje (objašnjavajući 64% varijanse u ponašanju ukupno).

Brojne studije su pokazale da farmeri donose različite upravljačke odluke u različitim fazama svog života. Mlađi farmeri sa velikim stadima i malo izdržavanih porodica spremniji su da učestvuju u programu iskorenjivanja, dok su stariji farmeri bez potomstva manje spremni da promene svoje sisteme upravljanja (39). Obrazovanje, iskustvo i kognitivne sposobnosti su varijable koje imaju značajan uticaj na proces donošenja odluka i često su povezane sa godinama donosioca odluka. Pored toga, pokazalo se da obrazovanje i obuka poboljšavaju i utiču na spremnost farmera da sprovedu promenu u praksi upravljanja (40). Poljoprivrednici koji su iskusili izbijanje bolesti u prošlosti će verovatnije koristiti izvore informacija i primenjivati više mera biološke bezbednosti trenutno na svojim farmama. Pored toga, farmeri koji su članovi programa zdravstvene zaštite goveda i ovaca verovatno će primeniti više mera biološke bezbednosti na svojim farmama.

Mala porodična gazdinstva igraju važnu ulogu u razvoju ruralne ekonomije, smanjujući siromaštvo među poljoprivrednicima, posebno u zemljama u razvoju. Međutim, ova kategorija proizvođača se tokom poslednje decenije suočila sa brojnim finansijskim, tehničkim i pravnim izazovima (41).

Prema Canu i Altugu (25), biosigurnost igra ključnu ulogu u sprečavanju zaraznih bolesti i povećanju produktivnosti farme. Oni su sproveli na ukupno 50 malih farmi muznih krava u Hataju u Turskoj, kako bi utvrdili tehničke i ekonomske rezultate farmi i ispitali povezanost između praksi biosigurnosti i socioekonomskih karakteristika proizvođača,

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koristeći kontrolnu listu koja se odnosi na 19 biosigurnosti. prakse, koristeći tehničke i ekonomske sisteme bodovanja prisustva i cene svake od praksi biosigurnosti. Ustanovili su da su najčešće korišćeni tretmani bolesnih životinja (98%), vakcinacija protiv najčešćih zaraznih bolesti (90%) i upotreba kreča za staje (86%), dok su testiranja na najčešće zarazne bolesti pre kupovina (10%) je korišćena po najnižoj stopi.

Autori su pronašli značajne razlike među grupama u pogledu nivoa obrazovanja (<.05), dohodovne klase (<.05) i veličine stada (<.01). Rezultati biosigurnosti bili su u značajnoj pozitivnoj korelaciji sa veličinom stada (<.05) i nivoom obrazovanja proizvođača (<.01). Postojale su statistički značajne veze između socioekonomskih karakteristika proizvođača i nekih praksi biosigurnosti. Autori su zaključili da programi obuke treba da imaju za cilj da promene stavove i percepciju malih proizvođača o lošim praksama biološke bezbednosti. Kako bi podstakli proizvođače da povećaju rezultate biosigurnosti, propisi koji se odnose na finansijsku podršku i kazne mogli bi biti veoma korisni i na regionalnom i na nacionalnom nivou.

Analiza stanja biosigurnosti na farmama mleka, goveda i ovaca u Srbiji različitog kapaciteta u diplomskim radovima studenata zootehnike koju su uradili Hristov i Stanković tokom poslednje decenije otkriva mnogo problema. Nivo biosigurnosti je uglavnom dobar ili veoma dobar ili retko dovoljan (prosečne ocene farmi su bile u rasponu od 3,85 do 1,49). Postoji ozbiljan nedostatak obuke i shodno tome, prilično nizak nivo svesti o rizicima biološke bezbednosti. To ukazuje da odgajivači moraju biti upoznati sa modelima širenja patogena i ulaznim vratima u pojedinu životinju ili populaciju. Poljoprivrednici dosta dobro vode računa o opštem zdravlju životinja (npr. mastitis, poremećaji kandži, problemi sa varenjem itd.), ali izgleda da nisu svesni rizika od zaraznih bolesti. Kontrola posetilaca je prilično liberalna, što je opšta situacija u Srbiji. Neophodne procedure se mogu obavljati mnogo upornije i odgovornije, posebno tokom leta, kada poljski radovi oduzimaju mnogo vremena poljoprivrednicima. Biosigurnosne procedure moraju biti ozbiljnije i određene približavanjem objektu zaštite.

OGRANIČENJA I OGRANIČENJA IMPLEMENTACIJE BIOSIGURNOSNIH PROCEDURA

Istorijski gledano, uloge i odgovornosti vlade, industrije i vlasnika životinja u vezi sa kontrolom bolesti bile su zasnovane na nizu pretpostavki koje nisu uvek rezultirale zajedničkim radom glavnih aktera. Barel (42) je postavio pitanje: „Šta proizvođači mogu da urade da se zaštite od epidemija bolesti?“ navodeći da tu industrija očigledno ima glavnu ulogu. Jedan od mogućih odgovora bio je rast nezavisnih inicijativa za biosigurnost među motivisanim farmerima ovaca i goveda i između farmera i njihovih veterinarskih savetnika u nekoliko regiona UK (1).

Toma i sar. (38) su zaključili da što je jači stav farmera prema biosigurnosti, veća je verovatnoća da će primeniti mere biosigurnosti na svojim farmama (naime da su mere biosigurnosti od suštinskog značaja za održavanje zdravog stada, da bi primena mera biosigurnosti mogla mnogo da uštedi poljoprivrednike novca i da su propisi o biosigurnosti dobri za zdravlje životinja). Ogromna većina veterinara smatra da je biosigurnost prioritet njihove profesije, iako ne smatraju da je njihova bezbednost ugrožena u svakodnevnoj praksi. Ovo bi moglo predstavljati pretnju po javno zdravlje pošto je seroprevalencija zoonoza znatno veća među veterinarima u ruralnim područjima (43). Anketa je istakla slabosti i oblasti za poboljšanje, posebno u vezi sa bio-isključivanjem (u vezi sa rizikom od unošenja bolesti) i biološkim zadržavanjem (u odnosu na rizik od prenošenja bolesti između stada). U slučaju da ne usvoje dobru praksu, veterinari bi mogli biti neuspešni u jednoj od svojih glavnih obaveza, da ograniče širenje bolesti u slučaju izbijanja, i da predstavljaju visok rizik za farmere jatrogenim putem prenošenja bolesti u prostorijama. Iako su veterinari izrazili različita ograničenja, moguća rešenja postoje i već su ih primenili neki veterinari, kao što je autonomni i mobilni sistem za dekontaminaciju ili odeća, čizme i/ili kutije za hirurški materijal namenjen farmi koje se ostavljaju u prostorijama.

Nedostatak finansijskih sredstava može biti važan faktor za male i srednje farmeree da (ne)usvoje određene mere biosigurnosti (44). Jednostavne i isplative mere biološke bezbednosti mogu se lako primeniti, uključujući informisanje posetilaca o ograničenjima pristupa, pravilno postupanje sa životinjama i obezbeđivanje da posetioci dezinfikuju ruke i obuću pre nego što uđu u objekte za uzgoj goveda. Pored toga, neophodno je imati određeni parking prostor za vozila i to treba naglasiti. Postupci čišćenja i dezinfekcije su od suštinskog

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značaja za sprečavanje širenja patogena, a posebno je potrebno očistiti i dezinfikovati sva vozila koja redovno ulaze u posed. Svakodnevno čišćenje i dezinfekcija kamiona zajedničkih za prevoz životinja su od ključne važnosti, iako ove mere mogu zahtevati značajna finansijska ulaganja ili rad zaposlenih da bi se te mere sprovele (24).

Slika 2. Predloženi konceptualni model koji objašnjava faktore koji utiču na sprovođenje mera biosigurnost i na belgijskim farmama goveda. U narandžastim poljima su dati faktori negativnog dejstva; u zelenim poljima su dati faktori pozitivnog dejstva (11) – pogledati englesku verziju rada

Renault i sar. (11) su istakli da nekoliko faktora mogu negativno uticati na sprovođenje mera biosigurnosti na farmama goveda. One se odnose i na stavove i svest farmera i na administrativne i pravne okolnosti. Na osnovu ovoga, može se dati nekoliko preporuka u cilju poboljšanja nivoa biosigurnosti na farmama goveda, kao što se može zaključiti na slici 2. Autori su istakli da postoji neodložna potreba za studijama troškovne efikasnosti zasnovanim na dokazima kako bi se identifikovala prioritetne mere biološke bezbednosti i dokazati poljoprivrednicima njihovu ekonomsku efikasnost (28). Prethodno je pomenuto da nivo percepcije veterinara o merama biosigurnosti značajno utiče na adekvatnu primenu dobrih praksi. Stoga je od suštinske važnosti dati biološkoj bezbednosti veću ulogu u programima veterinarske obuke i nastavnom planu i programu, i obezbediti odgovarajuću i progresivnu svest koja se pojavljuje kao deo kontinuirane edukacije koja se predlaže veterinarima (11).

Identifikovane mere moraju biti relevantne, adekvatne i izvodljive za potrebe farmera. Treba ih podeliti sa drugim zainteresovanim stranama kako bi se izbegle moguće kontradikcije (npr. uklanjanje elemenata „zelenog pojasa” koji okružuje farmu: grmlja i vegetacije radi kontrole vektora, dok ekološka politika promoviše prirodne žive ograde i sprečava uklanjanje vegetacije). Što se tiče pristupa prema konceptu jednog zdravlja, mere biosigurnosti treba smatrati sveobuhvatnim, kao „jedinstveni koncept za integraciju zdravlja ljudi, životinja, biljaka i životne sredine“ (45). Negativan uticaj nekih preventivnih tretmana na životnu sredinu ili zdravlje ljudi je dokumentovan u prošlosti, na primer razvoj (multi)rezistencije na lekove u vezi sa preventivnom upotrebom antibiotika u nekom sistemu intenzivnog uzgoja (46), kontaminacija životne sredine u vezi sa tretmanom životinja akaricidima (47) i negativnim efektima na korisne insekte posle upotrebe hemijskih larvicida u kontroli mesta za razmnožavanje vektora (48). Negativne posledice bi se mogle izbeći primenom pristupa Jednog zdravlja, povezujući oblasti zdravstva, poljoprivrede i zaštite životne sredine. Uzimanje u obzir prirodnih i društvenih nauka može olakšati prihvatanje mera biosigurnost i od strane stanovništva (44). Ovi aspekti su jasno uzeti u obzir Evropskim zelenim dogovorom (49), čije oblasti politike uključuju, između ostalog, biodiverzitet (mere za zaštitu ekosistema) i bezbednost hrane. Pored toga, efikasna obuka fokusirana na izabrane ciljeve i komunikacija sa farmerima bi se idealno trebalo odvijati od strane renomiranih izvora kao što su veterinari ili udruženja farmera, kako bi se promovisala biosigurnost i glavne mere biosigurnosti, znanje i svest zainteresovanih strana i pouzdano zakonodavstvo. Na kraju, ali ne i najmanje važno, dalje studije bi trebalo da pribave ubedljive dokaze o isplativosti mera biološke bezbednosti i da preporuče prioritetne mere za njihovu korisnost i koristi (16).

Uspešna primena preporučenih praksi upravljanja povezana je sa percepcijom rizika od strane farmera, uključujući prihvatljiv rizik i povezane posledice, kao i značaj određene mere biološke bezbednosti (11). Stoga, uočena efikasnost preporučenih smernica, izvodljivost i tehničko znanje o ovoj temi povećavaju verovatnoću usvajanja mera biosigurnosti u proizvodnim sistemima (24). Prema Oliveira-i i sar. (31), ukazano je na nedovoljno usvajanje biosigurnosti u mlečnim stadima. Ovo stvara stvarne rizike po zdravlje životinja i ljudi, osim što izaziva zabrinutost zbog ugrožene produktivnosti i dobrobiti životinja. Ipak, generalno, farmeri su upoznati sa važnim aspektima biološke bezbednosti. Uprkos tome, prisutni su faktori koji ograničavaju donošenje mera biosigurnost i.

ZAKLJUČCI

Opšta ocena sprovođenja mera biosigurnost i na farmama preživara širom sveta je da bi to moglo da se uradi mnogo bolje.

Iako je evidentno da svi učesnici u proizvodnji shvataju da imaju važnu ulogu u zdravlju stada i zaštiti proizvodnje od uzročnika patogena i izbijanja bolesti, njihovo znanje o procedurama i merama

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koje se moraju preduzeti često je veoma ograničeno. Postoji ozbiljan nedostatak obuke i shodno tome, prilično nizak nivo svesti o rizicima biološke bezbednosti. Poljoprivrednici su svesni neophodnosti dobrog zdravlja životinja, ali uglavnom misle na endemične bolesti poput mastitisa, poremećaja kandži, problema sa varenjem itd.); izgleda da nisu svesni rizika od zaraznih bolesti. To ukazuje da moraju biti upoznati sa modelima širenja patogena i ulaznim vratima u pojedinu životinju ili populaciju.

Mišljenje farmera je da bi vlada trebalo da ima veći doprinos u biosigurnost i; veterinari su kao glavnu prepreku videli sposobnost ili spremnost svojih klijenata da ulažu u mere biosigurnost i. Dodatni dokazi o efikasnosti i/ili potencijalnim ekonomskim koristima predloženih mera biološke bezbednosti na farmi treba da budu bolje prikazani. To bi dalo solidnu osnovu terenskim veterinarima i njihovim organizacijama da budu proaktivniji u promociji i širenju informacija o biosigurnost i, kako u direktnom kontaktu sa farmerima, tako iu organizovanoj obuci. Ovu važnu ulogu treba ostvariti u saradnji sa udruženjima poljoprivrednika i veterinarskim vlastima. Pravni okvir može biti od pomoći, kao i podsticajne mere za one koji aktivno i uspešno prihvataju koncept biosigurnost i u svom radu.

U mnogim slučajevima postoje problemi u vezi sa primenom procedura biosigurnosti, posebno u kontroli kretanja ljudi, životinja, vozila i opreme. Postupci se moraju izvoditi mnogo upornije i odgovornije, posebno tokom leta, kada poljski radovi oduzimaju mnogo vremena poljoprivrednicima. Biosigurnosne procedure moraju biti ozbiljnije i odlučnije primenjivane kao približavanje objektu zaštite.

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EFFECT OF SUBCLINICAL MASTITIS ON METABOLIC PROFILE IN DAIRY COWS

UTICAJ SUBKLINIČKOG MASTITISA NA METABOLIČKI PROFIL KRAVA

Jovan Stanojević*, Miodrag Radinović, Zorana Kovačević, Mihajlo Erdeljan, Jovan Spasojević, Tijana Kukurić

[1] Department of Veterinary medicine, Faculty of Agriculture, University of Novi Sad, Serbia
*Contact email: jovan2912@email.com

J.St. https://orcid.org/0009-0000-3328-6963, M.R. https://orcid.org/0000-0002-4390-4670, Z.K. https://orcid.org/0000-0002-0133-872X, M.E. https://orcid.org/0000-0002-4566-7584, J.Sp. https://orcid.org/0000-0001-5654-5796, T.K. https://orcid.org/0000-0003-2561-8555

APSTRACT

Mastitis is one of the most important disease in dairy cows, leading to signifficance economic losses. Subclinical mastitis is defined as inflammation of udder without any visible changes in the udder and milk. The aim of this study was investigate the influence of subclinical mastitis on the metabolic parameters in dairy cows. The study included 40 holstein Friesian cows with the same body score condition and in the same lactation phase. Divided into two groups of 20 each; subclinical mastitis and a control group of healthy cows. Cows with subclinical mastitis were detected by CMT (California mastitis test). Blood was sampled from the jugular vein, after morning milking and the concentration of glucose, NEFA, BHB, triglycerides, total protein, albumin, globulin, AST, ALT, ALP, LDH, calcium, magnesium and phosphorus ere determined. This study showed significant increase in concentration of NEFA, total protein, globulin and LDH in cows affected by subclinical mastitis compared to control group. On the other hand concentration of triglycerides and ALT was significant lower in cows affected by subclinical mastitis compared to healthy ones. Increase of the NEFA concentration and decrease in concentration of triglycerides can indicate on the increase of lipid mobilization with consequently possible development fatty liver or ketosis in cows, while increase in concentration of total protein and globulin indicate on inflammation. Beside, increase concentration of LDH in blood serum with increase of somatic cell count in the milk may be useful indicator for detect subclinical mastitis in dairy cows.

Key words: cows, subclinical mastitis, metabolic profile

SAŽETAK

Mastitis je jedna od najvažnijih bolesti kod muznih krava, koja dovodi do značajnih ekonomskih gubitaka. Subklinički mastitis se definiše kao zapaljenje vimena bez vidljivih promena u vimenu i mleku. Cilj ovog rada bio je da se ispita uticaj subkliničkog mastitisa na metaboličke parametre kod mlečnih krava. Studija je obuhvatila 40 holštajn frizijskih krava sa istim stanjem tela i u istoj fazi laktacije. Podeljeni u dve grupe od po 20; subklinički mastitis i kontrolna grupa zdravih krava. Krave sa subkliničkim mastitisom su otkrivene CMT (kalifornijski mastitis test). Uzeta je krv iz jugularne vene, nakon jutarnje muže i utvrđena koncentracija glukoze, NEFA, BHB, triglicerida, ukupnih proteina, albumina, globulina, AST, ALT, ALP, LDH, kalcijuma, magnezijuma i fosfora. Ova studija je pokazala značajno povećanje koncentracije NEFA, ukupnog proteina, globulina i LDH kod krava obolelih od subkliničnog mastitisa u poređenju sa kontrolnom grupom. Sa druge strane, koncentracija triglicerida i ALT je bila značajno niža kod krava obolelih od subkliničnog mastitisa u odnosu na zdrave. Povećanje koncentracije NEFA i smanjenje koncentracije triglicerida može ukazivati na povećanje mobilizacije lipida sa posledično mogućim razvojem masne jetre ili ketoze kod krava, dok povećanje koncentracije ukupnog proteina i globulina ukazuje na upalu. Osim toga, povećanje koncentracije LDH u krvnom serumu sa povećanjem broja somatskih ćelija u mleku može biti koristan indikator za otkrivanje subkliničkog mastitisa kod mlečnih krava.

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Ključne reči: krave, subklinički mastitis, metabolički profil

INDRODUCTION

Mastitis is one of the most important diseases in the dairy cows, leading to signifficance economic losses associated with the decreased milk production and quality, increased rate of culling and higher cost of veterinary treatment (1-3). Mastitis represents inflammatory reaction of the mammary gland as response to the injury or infection, caused by different causative agents (4). On the basis of visible signs of udder inflammation, mastitis could be divided in subclinical and clinical mastitis (5). Subclinical mastitis is harder for diagnostic, due to absence of visible signs and mostly remains unnoticed by the farmer. Additionally, subclinical mastitis can represent constant source of infection for other animals in the herd. For this reason, subclinical mastitis should be detected by specific tests are performed in milk samples. More advanced techniques are required for subclinical mastitis detection, including somatic cell evaluation, plate-culture methods, pH, electrical conductivity, enzymatic activity, molecular diagnostic tools, and biosensors (6). Subclinical mastitis leads to damage to the parenchyma of the mammary gland, which results in changes in the chemical composition of milk. Beside, subclinical mastitis leads to changes in metabolic parameters in the blood serum (7). It appears as a consequence cytokines released from immune cells. These cytokines cause difference changes in the metabolism of the cows. Furthermore, these metabolic changes can lead to various metabolic diseases in the cows. On the other hand, metabolic diseases such as ketosis could be cause the subclinical or clinical mastitis, because the ketone bodies impair the activity of immune cells and mammary gland defense (8). For this reason, the aim of this study was determining influence of subclinical mastitis on metabolic adaptation in dairy cows.

MATERIAL AND METHOD

This study was performed on the dairy farm in the republic of Serbia. The study included 40 holstein Friesian cows with the same body score condition and in the same lactation phase. Divided into two groups of 20 each; subclinical mastitis and a control group of healthy cows. Blood serum metabolic profiles were measured using a Rayto spectrophotometer (Rayto, Shenzhen, China). Metabolic profile was included concentration of glucose, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), triglyceride, total protein, albumin, globulin, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), calcium (Ca), magnesium (Mg) and phosphorus (P). All analyses were performed at the Laboratory of Pathophysiology, Department of Veterinary Medicine, University of Novi Sad. All the obtained results were summarized by Microsoft Office Excel (v2019) and processed by the application of Statsoft Statistica (v12.5) (Tulsa, Oklahoma).

RESULTS AND DISCUSSION

Table 1 shows metabolic parameters in cows affected by subclinical mastitis and healthy cows, and statistically significance difference in metabolic parameters between these groups of cows. This study showed significant increase in concentration of NEFA, total protein, globulin and LDH in cows affected by subclinical mastitis compared to control group. On the other hand concentration of triglycerides and ALT was significant lower in cows affected by subclinical mastitis compared to healthy ones. Increase of the NEFA concentration and decrease in concentration of triglycerides can indicate on the increase of lipid mobilization with consequently possible development fatty liver or ketosis in cows, while increase in concentration of total protein and globulin indicate on inflammation. Beside, increase concentration of LDH in blood serum with increase of somatic cell count in the milk may be useful indicator for detect subclinical mastitis in dairy cows.

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Table 1. Metabolic characteristics of healthy cows and cows with subclinical mastitis

	Subclinical mastitis	Healthy cows	Statistical significance
Glucose(mmol/L)	1.92 ± 0.34	2.03 ±0.48	NS
NEFA(mmol/L)	0.44 ± 0.18	0.34 ± 0.08	P < 0.05
BHB(mmol/L)	0.35 ± 0.16	0.31 ± 0.09	NS
Triglycerides (mmol/L)	0.51 ± 0.34	0.70 ± 0.29	P < 0.05
Total protein (g/L)	66.1 ±10.23	59.46 ± 6.64	P < 0.01
Albumin (g/L)	27.35 ± 3.54	29.51 ± 3.03	NS
Globulin (g/L)	38.74 ± 8.93	29.69 ± 4.74	P < 0.01
AST (U/L)	76.76 ± 40.27	66.53 ±14.17	NS
ALT (U/L)	27.04 ± 6.85	36.79 ±8.94	P < 0.01
ALP (U/L)	39.56 ± 14.21	35.33 ±9.07	NS
LDH (U/L)	1366.05 +/-203.69	984.68 ± 152.14	P < 0.01
Ca (mmol/L)	2.13 +/- 0 .33	2.02 ± 0.36	NS
P (mmol/L)	2.53 +/- 0.71	2.38 ± 0.47	NS
Mg (mmol/L)	0.77 +/- 0.33	0.66 ± 0.35	NS

*NS – non-statistically significant difference; p<0.01, P<0.05 statistically significant differences

Bovine mastitis remains a significant global concern, causing economic losses in the dairy industry due to reduced milk production, increased treatment expenses, lower fertility, and elevated culling of affected animals (9, 10). Subclinical mastitis has negative effect on the economy of dairy farmers due to directly affect on the milk quality and quantity (11). Inflammatory response of the mammary gland can lead to damage to the parenchyma of mammary gland and different changes in metabolic profile. This response includes increase level of cytokines, which have key role in hemotaxis, activation of immune cells and increase of phagocytic activity of macrophages and neutrophils (12). Beside, these cytokines can causing lipomobilisation of the NEFA from perypherial adipose tissue (13). This fact could be explain significance increase of NEFA concentration in cows affected by subclinical mastitis in compare to healthy one. Furthermore, high level of NEFA in serum may be significant predisposition for develop fat liver and ketosis in cows. For these reasons, early detection of subclinical mastitis in dairy herd is very important for animal health.

In current study, significance difference in concentration of NEFA, triglycerides, total protein, globulin, ALT and LDH were recorded between cows affected by subclinical mastitis and healthy cows. Significant higher concentration of NEFA, total protein, globulin and LDH was recorded in cows affected by subclinical mastitis in compare to healthy ones. On the other hand, concentration of triglycerides and ALT was significant lower in cows affected by subclinical mastitis in compare to healthy animals. The increase of NEFA concentration could be explained by greater of mobilization from adipose tissue, which is caused by release of different cytokines in inflammatory response (13). Schwegler et al. (2013) suggested that cows with low glucose and higher NEFA in the prepartum were more susceptible for mastitis in the early postpartum, probably due to low immune function associated to a more negative energy balance (14). Beside, cytokines released from activated immune cells primarily macrophages stimulate the production of positive acute-phase proteins (e.g., haptoglobin) and reduce the synthesis of negative acute-phase proteins (e.g., albumin) in the liver (13). This fact could be explains obtained results, which show the decrease of albumin and increase of total protein and globulin in cows affected by subclinical mastitis. Beside, activated bovine plasma cells release the immunoglobulines (15), which belong at globulins. Matei et al. (16) recorded significant increase of total serum protein and globulin in cows with subclinical form of mastitis in compare to healthy cows. They suggest the increase proteins and globulin in the blood of cows indicate an activation of immune response following infection of the mammary gland (16). Beside, Ali et al. (17) found the higher concentration of total protein and lower concentration of globulin in cows with subclinical mastitis compared to healthy cows. Furthermore, these mentioned authors didn't find the significance difference in albumin concentration between sick and healthy cows and that's in accordance with this study (17).

In this study was observed significant lower concentration of triglycerides in cows affected by subclinical mastitis compared to healthy ones. This could be as a consequence increase of lipid mobilization and decrease of functional activity of the liver. In contrary to these results, Ali et al. (17) didn't find significant difference in triglyceride concentration between cows with subclinical mastitis and healthy ones.

Lactate dehydrogenase (LDH) is widely distributed enzyme in cells of various living systems where it is involved in carbohydrate metabolism catalyzing interconversion of lactate and pyruvate with NAD^+/NADH coenzyme system (18). Changes in plasma or serum enzymes are useful indicators of tissue damage in many diseases (18). The LDH in milk originates from the damaged udder epithelial cells or from the leukocytes in milk (18). Increase concentration of LDH in milk lead to increase the concentration of this enzyme in blood. These could be explain the results obtained in present study, where significantly higher levels of LDH are determined in the blood serum from cows affected by subclinical mastitis compared to healthy ones. These results are in accordance with other authors (19).

CONCLUSION

The healthy cows and mammary glands are the most important conditions for the successful of milk production. Subclinical mastitis leads to significant changes in metabolic profile of dairy cows. These changes arise as a consequent inflammatory response of the mammary gland. Increase of the NEFA concentration and decrease in concentration of triglycerides can indicate on the increase of lipid mobilization with consequently possible development fatty liver or ketosis in cows, while increase in concentration of total protein and globulin indicate on inflammation. Beside, increase concentration of LDH in blood serum with increase of somatic cell count in the milk may be useful indicator for detect subclinical mastitis in dairy cows.

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REFFERENCES

1. *Mbindyo C.M., Gitao G.C., Mulei C.M. Prevalence, etiology, and risk factors of mastitis in dairy cattle in Embu and Kajiado Counties, Kenya. Veterinary medicine international 2020,2020.*
2. *Aqib A.I., Muneer A., Shafeeq M., Kirn N. Economic Impacts of Clinical and Sub Clinical Mastitis on Dairy Farms. Veterinary Science Research 2021, 3(2):31-9.*
3. *Hadrich J., Wolf C., Lombard J., Dolak T. Estimating milk yield and value losses from increased somatic cell count on US dairy farms. Journal of Dairy science 2018,101(4):3588-96.*
4. *Chauhan R.S., Malik Y.S., Saminathan M., Tripathi B.N. Immunopathology of Mastitis. Essentials of Veterinary Immunology and Immunopathology 2024, 373-403.*
5. *Cobirka M., Tancin V., Slama P. Epidemiology and classification of mastitis. Animals 2020, 10(12):2212.*
6. *Thompson J., Everhart Nunn S.L., Sarkar S., Clayton B. Diagnostic Screening of Bovine Mastitis Using MALDI-TOF MS Direct-Spotting of Milk and Machine Learning. Veterinary Sciences 2023, 10(2):101.*
7. *Stanojević J., Kreszinger M., Radinović M., Kladar N., Tomanić D., Ružić Z., et al. Assessment of Mastitis Patterns in Serbian Dairy Cows: Blood Serum Metabolic Profile and Milk Composition Parameters. Pathogens 2023, 12(11):1349.*
8. *Swartz T.H., Bradford B.J., Mamedova L.K. Connecting metabolism to mastitis: Hyperketonemia impaired mammary gland defenses during a Streptococcus uberis challenge in dairy cattle. Frontiers in Immunologyn2021, 12:700278.*
9. *Azooz M., El-Wakeel S.A., Yousef H. Financial and economic analyses of the impact of cattle mastitis on the profitability of Egyptian dairy farms. Veterinary World 2020,13(9):1750.*
10. *Kitade Y., Tsukano K., Miyamoto Y., Suzuki K. Mastitis causes negative reproduction performance similar to genital diseases. Research in Veterinary Science 2022,153:35-44.*

ISBN 978-86-7520-611-8

11. *Ranasinghe R., Deshapriya R., Abeygunawardana D., Rahularaj R, Dematawewa C. Subclinical mastitis in dairy cows in major milk-producing areas of Sri Lanka: Prevalence, associated risk factors, and effects on reproduction. Journal of dairy science 2021,104(12):12900-11.*
12. *Kawecka-Grochocka E., Zalewska M., Rzewuska M., Kościuczuk E., Ząbek T., Sakowski T., et al. Expression of cytokines in dairy cattle mammary gland parenchyma during chronic staphylococcal infection. Veterinary Research 2021,52:1-12.*
13. *Mezzetti M., Bionaz M., Trevisi E. Interaction between inflammation and metabolism in periparturient dairy cows. Journal of animal science 2020, 98(Supplement_1):S155-S74.*
14. *Schwegler E., Schneider A., Montagner P., Acosta D.A.V., Pfeifer L.F.M., Schmitt E., et al. Predictive value of prepartum serum metabolites for incidence of clinical and subclinical mastitis in grazing primiparous Holstein cows. Tropical animal health and production 2013,45:1549-55.*
15. *Gondaira S., Nishi K., Tanaka T., Yamamoto T., Nebu T., Watanabe R., et al. Immunosuppression in cows following intramammary infusion of Mycoplasma bovis. Infection and immunity 2020, 88(3):10.1128/iai. 00521-19.*
16. *Matei S.T., Groza I., Andrei S., Bogdan L., Ciupe S., Petrean A. Serum metabolic parameters in healthy and subclinical mastitis cows, 2010.*
17. *Ali A., Mir B.A., Bhat R.R., Baba O.K., Hussain I., Rashid S.M., et al. Metabolic profiling of dairy cows affected with subclinical and clinical mastitis. J Entomol Zool Stud. 2017, 5(6):1026-8.*
18. *Klein R., Nagy O., Tóthová C., Chovanová F. Clinical and diagnostic significance of lactate dehydrogenase and its isoenzymes in animals. Veterinary medicine international 2020, 2020.*
19. *Saleh N., Allam T.S., Omran A., Abdelfattah A.M. Evaluation of Changes in Hemato-Biochemical, Inflammatory, and Oxidative Stress Indices as Reliable Diagnostic Biomarkers for Subclinical Mastitis in Cows. Alexandria Journal for Veterinary Sciences 2022, 72(2).*

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Article
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SPORADIC LEUKOSIS IN CATTLE

SPORADIČNA LEUKOZA KOD GOVEDA

Jože Starič*, Lena Veren Geč, Rok Marzel, Jožica Ježek

Clinic for Reproduction and Large Animals – Section for Ruminants, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia
*Correspondence: joze.staric@vf.uni-lj.si

J.S. https://orcid.org/0000-0002-5156-5277, R.M. https://orcid.org/0009-0000-2753-3374, J.J. https://orcid.org/0000-0002-3753-5944

ABSTRACT

Sporadic bovine leukosis (SBL) is a rare neoplastic disease in cattle. It represents a unique challenge for veterinary medicine due to its sporadic occurrence, peculiar clinical presentation and unclear etiology. SBL manifests in four forms: juvenile lymphosarcoma, adult multicentric leukosis, thymic lymphosarcoma and cutaneous lymphosarcoma. In contrast to enzootic bovine leukosis, which is caused by the bovine leukemia virus (BLV), there is no viral link in sporadic cases. They are thought to be caused by multifactorial interactions between genetic predisposition and environmental factors (e.g. carcinogens or other triggers). The diseases are not contagious. The aim of this paper is to review the current knowledge of SBL, focusing on the clinical presentation, diagnostic challenges and potential risk factors. Although sporadic leukosis accounts for a smaller proportion of leukosis cases in cattle compared to BLV-induced leukosis, the impact on affected individuals is severe. SBL must be differentiated from BLV-induced leukosis (absence of BLV antibodies in the blood and absence of BLV in the tissue), which is a reportable disease in the European Union. SBL usually affects younger cattle, but adult animals can also be affected. Adult multicentric leukosis clinically resembles enzootic bovine leukosis with multicentric lymphoproliferative foci and lymphadenopathy, but the affected animals are negative for BLV antibodies. The diseases are progressive and lead to the death of the animals. Diagnostic confirmation of SBL often requires a combination of histopathologic examination, immunohistochemistry and molecular analyses to differentiate it from other neoplastic diseases in cattle. Risk factors such as exposure to certain environmental toxins or genetic susceptibility are thought to play a role in the development of sporadic leukosis. Further research efforts are needed to elucidate the mechanisms underlying the development of SBL and to develop effective prevention and control strategies to control this sporadic but usually lethal disease in cattle population.

Keywords: multicentric leukosis, lymphosarcoma, thymic lymphosarcoma, cutaneous lymphosarcoma, bovine

SAŽETAK

Sporadična leukoza goveda (SBL) je retka neoplastična bolest goveda. Predstavlja jedinstven izazov za veterinu zbog svoje sporadične pojave, osobene kliničke slike i nejasne etiologije. SBL se manifestuje u četiri oblika: juvenilni limfosarkom, multicentrična leukoza odraslih, limfosarkom timusa i kožni limfosarkom. Za razliku od enzootske leukoze goveda, koju izaziva virus goveđe leukemije (BLV), ne postoji virusna veza u sporadičnim slučajevima. Smatra se da su uzrokovane multifaktorskim interakcijama između genetske predispozicije i faktora životne sredine (npr. kancerogeni ili drugi okidači). Bolesti nisu zarazne. Cilj ovog rada je da se sagledaju dosadašnja znanja o SBL, fokusirajući se na kliničku prezentaciju, dijagnostičke izazove i potencijalne faktore rizika. Iako sporadična leukoza čini manji udeo slučajeva leukoze kod goveda u poređenju sa leukozom izazvanom BLV, uticaj na obolele osobe je ozbiljan. SBL se mora razlikovati od leukoze izazvane BLV (odsustvo BLV antitela u krvi i odsustvo BLV u tkivu), što je bolest koja se prijavi u Evropskoj uniji. SBL obično pogađa mlađa goveda, ali mogu biti pogođene i odrasle životinje. Multicentrična leukoza odraslih klinički podseća na enzootsku leukozu goveda sa multicentričnim limfoproliferativnim žarištima i limfadenopatijom, ali su obolele životinje negativne na BLV antitela. Bolesti su progresivne i dovode do uginuća životinja. Dijagnostička potvrda SBL često zahteva kombinaciju histopatološkog pregleda, imunohistohemije i molekularne analize da bi se razlikovala od drugih neoplastičnih bolesti goveda. Smatra se da faktori rizika kao što su izloženost određenim toksinima iz životne sredine ili genetska osetljivost igraju ulogu u razvoju sporadične leukoze. Potrebni su dalji istraživački napori da bi se razjasnili mehanizmi koji leže u osnovi razvoja SBL i da se razviju efikasne strategije prevencije i kontrole za kontrolu ove sporadične, ali obično smrtonosne bolesti u populaciji goveda.

Ključne reči:multicentrična leukoza, limfosarkom, multicentric leukosis, lymphosarcoma, timusni limfosarkom, kutani limfosarkom, goveda.

INTRODUCTION

Bovine leukosis or bovine lymphosarcoma (other names are malignant lymphoma and leukemia) is a neoplastic disease of cattle characterized by a progressive and malignant growth of lymphoid tissue in the lymph nodes and other organs. The disease is defined as sporadic or endemic / enzootic according to the frequency of occurrence, the age of the animal in which it occurs, the organ system(s) affected and the causative agent (Hendrick, 2002).
It occurs sporadically in calves as generalized lymphadenopathy (calf or juvenile form), thymic leukosis in cattle between 6 months and 2 years of age (thymic or adolescent form) and in 1- to 3-year-old cattle as the cutaneous form. The causative agent of the sporadic forms has not yet been discovered; it appears that they are not infectious in nature. There are approximately 1.55 cases of sporadic forms of leukosis per 10,000 animals that are not infected with the formal bovine leukemia virus (BLV), which was renamed in 2023 into Deltaretrovirus bovleu. The most common form of leukosis is enzootic bovine leukosis (EGL) in adult cattle older than 2 years, which is caused by infection with Deltaretrovirus bovleu, from the family Retroviridae. It can affect several organ systems simultaneously. Enzootic bovine leukosis is also the most common neoplastic disease in cattle (Hendrick, 2002; Nasir, 2005).

Sporadic lymphosarcomas

Calf or juvenile form

The prevalence of this form is very low and multiple cases in the same herd are very rare (Hendrick, 2002). It occurs uniformly throughout the world. The cause of the disease is unknown and does not appear to be related to the EGLvirus. Experimentally, also the disease cannot be transmitted to healthy calves. There are no differences between the sexes in terms of morbidity, but dairy breeds are said to be more prone to developing the disease (Dubreuil et al., 1998; Frischkorn et al., 2022; Hendrick, 2002).
Calves between 3 and 6 months of age are affected most commonly, but in rare cases it has also occurred in younger calves from 2 weeks of age or in older cattle up to 3 years of age. There are even known cases where sick calves have already been born(Frischkorn et al., 2022; Hendrick, 2002).
The main complaints for seeking veterinary help for sick animals is lethargy, weight loss, weakness despite a good appetite or lymphadenopathy. The disease usually develops rapidly within a week.
Clinical examination shows a generalized bilateral enlargement of the lymph nodes. Rarely, the lymph node enlargement is limited to a specific anatomical region. The enlarged lymph nodes are hard, smooth and painless and may be the size of a hen's egg or even larger. The mucous membranes are usually pale due to anemia. Heart rate and respiratory rate are accelerated. Coughing and abnormal breathing sounds may be present. Rarely, the animals may also have an enlarged liver, increased body temperature, rumen tympany, diarrhea, ataxia or paralysis. The affected animals usually die within 2 to 8 weeks after the first clinical signs appear (Dubreuil et al., 1998; Frischkorn et al., 2022; Hendrick, 2002).
The hematological values of affected animals show microcytic and hypochromic anemia, low hematocrit, thrombocytopenia and leukocytosis due to lymphocytosis. An increased myeloid/erythroid ratio with severe neoplastic infiltration may be seen in the bone marrow. Neoplastic changes can also be

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found in various organs, e.g. in the spleen, liver, heart, kidneys, pancreas, uterus and thymus (though less pronounced than in the thymic form). Neoplastic infiltrations may also be found subperiosteally, including in vertebrae, where often comes to pressing on the spinal cord resulting in paresis. Necrotic foci may also be present in the bones (Dubreuil et al., 1998; Frischkorn et al., 2022; Hendrick, 2002).
The disease has also been diagnosed a few times by the faculty of Veterinary faculty.

Thymic or adolescent form

This form of lymphosarcoma is extremely rare. It occurs most frequently in cattle between 6 and 24 months of age, but also rarely in newborn animals and animals up to 4 years of age6. Meat breeds are predisposed to the occurrence of this form of the disease (Nasir, 2005). In France, numerous cases of thymic lymphosarcoma have been detected in 5 regions and a large number of affected calves were found to come from the same bull, suggesting that the disease may be hereditary7.
The clinical signs are the result of formation that occupy the space on the neck and chest. Most commonly, owners seek veterinary advice because of the swelling in front of the sternum, from the entrance to the chest cavity to the intermandibular space, which is hard and edematous. This often leads to weight loss, rumen tympany (due to pressure on the esophagus) and dysphagia. Generalized lymphadenopathy is rare, but the superficial cervical lymph nodes are often enlarged. The enlarged thymus cannot usually be palpated, but in dissection it is visible in the thoracic cavity. The jugular vein is often tense and does not pulsate. The heartbeat may be less audible. The animal may also be tachycardic, dyspneic, may cough and show signs of respiratory distress(Nasir, 2005).
In some cases, it metastasizes, including to the spinal canal, where it causes compression of the spinal cord(Hendrick, 2002; Nasir, 2005).
The hematological findings are often unremarkable, anemia is present only occasionally and so is lymphocytosis.
It takes 2 to 9 weeks from the diagnosis of the disease to the death of the affected animal, although the disease can cause a deterioration in health months before diagnosis. The disease can quickly lead to death due to acute rumen tympany (Nasir, 2005).

Skin form

This form of lymphosarcoma is also rare, but more prevalent than juvenile and adolescent forms. It occurs in cattle aged 1 to 4 years and is characterized by numerous tumor formations of 1 to 5 cm in diameter on the skin of the neck, back, hips, lower part of the tail, perineum and thighs. From the medical history, we often learn that such changes on the skin may appear for 1 to 3 months, then regress and then reappear. Initially, amber-colored scabs appear on the skin lesions, later gray-white, dry deposits, the hair falls out, then a depression appears in the middle. The lesions may also ulcerate and feel painful. At the beginning they have an elastic consistency, later they are firm-elastic. As a rule, the peripheral lymph nodes are also greatly enlarged and firm. Other clinical signs depend on which organ system is also affected. The animals are often anemic and have lymphocytosis, often with atypical lymphocytes. The disease always ends in death (Klinkon & Černe, 2006; Kureljušić et al., 2019; Loh, 2007; Schweizer et al., 2003).
On pathoanatomical examination, various organs and organ systems can be affected by leukotic changes. The skin changes are grayish in cross-section. The skin is heavily infiltrated with lymphoid cells (Hendrick, 2002; Hugoson, 1970; Klinkon & Černe, 2006; Kureljušić et al., 2019; Loh, 2007; Schweizer et al., 2003). The last time we had a case of cutaneous leukosis at the Clinic for Ruminants was in 2022 (archive of the clinic's patient files).

Photo 1: Skin leucosis in Brown cow (note cutaneous nodules, which were firm, hairless and sometimes covered with hemorrhagic crusts) (Photo: J Starič)

Sporadic multicentric lymphosarcoma in adult cattle

Presents similarly as enzootic bovine leukosis, caused by BLV infection. However, is such cases BLV virus cannot be detected by ether PCR from blood or affected tissues and the affected animal is also seronegative for BLV antibodies. The disease is sporadic (Freick et al., 2016).

CONCLUSION

Diagnostic confirmation of SBL often requires a combination of histopathologic examination, immunohistochemistry and molecular analyses to differentiate it from other neoplastic diseases in cattle. Cases of SBL also require testing for BLV, to exclude possibility of enzootic bovine leukosis. Especially in countries that are officially free of enzootic bovine leukosis (EBL), such diagnostics is mandatory by regulations. Risk factors such as exposure to certain environmental toxins or genetic susceptibility are thought to play a role in the development of sporadic leukosis. Further research efforts are needed to elucidate the mechanisms underlying the development of SBL and to develop effective prevention and control strategies to control this sporadic but usually lethal disease in cattle population.

LITERATURE

1. *Dubreuil P., Lanevschi A., Perrone M. A., Desnoyers M. Atypical sporadic lymphosarcoma in a 7-month-old Holstein heifer. Canadian Veterinary Journal 1998, 39(7), 431–433.*
2. *Freick M., Lapko L., Neubert M., Hardt M., Behn H., Passarge O., SchöNiger S. Sporadische kutane T-Zell-Leukose mit Beteiligung von Lymphknoten und inneren Organen bei einer Holstein-Kuh. Tierarztliche Praxis Ausgabe G: Grosstiere – Nutztiere 2016, 44(1), 39–45. https://doi.org/10.15653/TPG-150514*
3. *Frischkorn M., Fortelny M., Ciurkiewicz M., Krüger J., Jahn-Falk, D. Multicentric B cell lymphoma in an 8-week-old calf. Tierarztliche Praxis Ausgabe G: Grosstiere – Nutztiere 2022, 51(2), 109–115. https://doi.org/10.1055/a-2030-6701*
4. *Hendrick S.H. Atypical sporadic bovine leukosis in a beef feedlot heifer. Canadian Veterinary Journal 2002, 43(8), 617–619.*
5. *Hugoson G. Tissue Culture Studies in Bovine Leukosis. Acta Veterinaria Scandinavica 1970, 11(1), 103–113. https://doi.org/10.1186/BF03548007*
6. *Klinkon M., Černe M. Cutaneous T-cell lymphoma in a heifer with increased serum lactate dehydrogenase activity. https://doi.org/10.1111/j.1939-165X.2006.tb00120.x, 2006.*
7. *Kureljušić B., Maksimović S., Vujinović S., Savić B., Milićević V., Veljović L., Jezdimirović N., Žutić J. Cutaneous lymphoma in a cow - a case report. Veterinarski Glasnik 2019, 73(1), 50–56. https://doi.org/10.2298/VETGL180904004K*
8. *Loh, C. C. (2007). Atypical presentation of cutaneous T-cell lymphosarcoma in a 19-month-old Holstein heifer. Canadian Veterinary Journal, 48(3), 309–313.*
9. *Nasir K. S. Sporadic juvenile thymic lymphoma in a 6-month-old Holstein heifer. Canadian Veterinary Journal 2005, 46(9), 831–833.*
10. *Schweizer G., Hilbe M., Braun U. Clinical, haematological, immunohistochemical and pathological findings in 10 cattle with cutaneous lymphoma. The Veterinary Record 2003, 153(17), 525–528. https://doi.org/10.1136/VR.153.17.525*

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Review
Pregledni rad

ANTHELMINTIC RESISTANCE IN GASTROINTESTINAL NEMATODES OF SHEEP: CURRENT SITUATION AND NOVEL STRATEGIES

ANTIHELMINTIČKA REZISTENCIJA KOD GASTROINTESTINALNIH NEMATODA OVACA: AKTUELNA SITUACIJA I NOVE STRATEGIJE

Filip Štrbac[a*], Dragica Stojanović[b]

[a]Institute for Multidisciplinary Research, University of Belgrade, Serbia
[b]Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad, Serbia
*Contact author: filip.strbac@imsi.bg.ac.rs

F.Š. https://orcid.org/0000-0002-2666-7992, D.S. https://orcid.org/0000-0003-4781-2623

ABSTRACT

Gastrointestinal nematodes nowadays represent a major obstacle to sustainable sheep farming due to their negative effect on animal health, welfare and productivity. Commercial drugs such as benzimidazoles, macrocyclic lactones and imidazothiazoles have been used with success in previous decades to control these parasites. However, their irrational application has led to the development of anthelmintic resistance and large economic losses, while the situation is expected to further deteriorate in the future due to the spread of resistance and the emergence of multi-resistant nematode strains. Thus, monitoring is of key importance, which involves the application of various *in vitro* and *in vivo* tests, as well as modern molecular methods in order to early detect the development of resistance and monitor the situation in a certain area. In addition, the problem of the exclusive application of chemical preparations is also reflected in the residues in meat and milk, as well as in the environment. This poses a risk to various organisms, including humans. For these reasons, it is necessary to define new strategies, which are based on the rational application of anthelmintics in terms of targeted treatments, targeted selective treatments, but also combination and rotation of preparations. The introduction of alternative methods into practice, such as phytotherapy, i.e. the use of plant preparations such as extracts and essential oils, direct and indirect biological control, development of vaccines, genetic selection of naturally resistant animals with appropriate management of pastures and nutritional status of animals are also needed, all with the aim of reducing application of commercial drugs. This implies an integrated approach to the control of gastrointestinal nematodes, which is the basis of future treatments.

Keywords: gastrointestinal nematodes, anthelmintic resistance, rational use of anthelmintics, alternative strategies, integrative approach

SAŽETAK

Gastrointestinalne nematode danas predstavljaju glavnu prepreku održivom ovčarstvu zbog njihovog negativnog efekta na zdravlje, dobrobit i produktivnost životinja. Komercijalnu preparati poput benzimidazola, makrocikličnih laktona i imidazotiazola su se sa uspehom koristili prethodnih decenija u kontroli ovih parazita. Međutim, njihova neracionalna primena je dovela do razvoja antihelmintičke rezistencije i velikih ekonomskih gubitaka, pri čemu se u budućnosti očekuje dodatno pogoršanje situacije zbog širenja rezistencije i pojave multirezistentnih sojeva nematoda. Zbog toga je monitoring od ključne važnosti, a koji podrazumeva primenu različitih *in vitro* i *in vivo* testova, kao i savremenih molekularnih metoda u cilju rane detekcije razvoja rezistencije i praćenja situacije na određenom području. Pored toga, problem isključive primene hemijskih preparata se ogleda i u ostacima rezidua u mesu i mleku, kao i životnoj sredini. To predstavlja rizik za različite organizme, uključujući i ljude. Iz ovih razloga je potrebno

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definisati nove strategije, koje se baziraju na racionalnoj primeni antihelmintika u smislu ciljanih tretmana, ciljanih selektivnih tretmana, ali i kombinacije i rotacije preparata. Od izuzetnog značaja je i uvođennje alternatnvih metoda u praksu poput fitoterapije odnosno upotrebe biljnih preparata poput ekstrakta i etarskih ulja, direktne i indirektne biološke kontrole, razvoja vakcina, genetske selekcije prirodno otpornih životinja uz odgovarajuće upravljanje pašnjacima i nutritivni status životinja, a sve u cilju smanjenja primene komercijalnih preparata. To podrazumeva integrisan pristup kontrole gastrointestinalnih nematoda, koji predstavlja osnovu budućih tretmana.

Ključne reči: gastrointestinalne nematode, antihelmintička rezistencija, racionalna primena antihelmintika, alternativne strategije, integrisan pristup

INTRODUCTION

The small ruminant industry today faces several problems that hinder sustainable production. One of the main obstacles are gastrointestinal nematodes (GINs), which are widespread in the world (1). They parasitize in different parts of the gastrointestinal tract, including the abomasum, small and large intestine, and usually have a direct life cycle. The prevalence and distribution of GIN species depends on geographical and climatic conditions, with *Haemonchus* spp. and *Cooperia* spp. being present in subtropical and tropical regions, *Teladorsagia* spp. and *Nematodirus* spp. in temperate regions, while *Trichostrongylus* spp. are present worldwide (2). In addition to these, *Chabertia* spp., *Oesophagostomum* spp. and *Bunostomum* spp., *Marshallagia* spp., *Strongyloides* spp. and *Trichuris* spp. also occur in Serbia (3,4). Therefore, GINs cause high economic losses in various countries and regions, including the Mediterranean region (5-8).

The extent of GIN infections and their negative effects depend on several factors, such as the age of the animal, the breed, the type of parasite involved and the degree of parasitic infection (8). Although these infections are usually subclinical, they still significantly affect the production of meat, milk and wool (5). With a high worm burden, GINs cause clinical infections with signs of anaemia, diarrhoea, anorexia and edema, which can even lead to animal death (9-11). In addition to the effects on animal health, productivity, fertility and welfare, the costs of anthelmintic treatments also contribute to the negative economic impact (6,12). Due to the various modes of causation, the total economic losses caused by GINs are difficult to quantify precisely, but are estimated to be in the hundreds of millions of EUR (6). In addition, GIN infection is predicted to increase the intensity of greenhouse gas emissions in sheep farms by up to 30 % (13).

THE EXCLUSIVE USE OF COMMERCIAL ANTHELMINTICS AS AN UNSUSTAINABLE APPROACH

Commercial anthelmintics are now the main method of controlling GINs in sheep (14,15). These mainly include benzimidazoles, BZ (e.g. albendazole, fenbendazole), macrocyclic lactones, ML (e.g. ivermectin) and imidazothiazoles, IMZ (e.g. levamisole). These drugs have different modes of action, as benzimidazoles bind to the alpha subunit of the β-tubulin protein, ML bind selectively and irreversibly to the subunits of chlorion channels that are activated by various neurotransmitters, while IMZ act selectively as cholinergic agonists (nicotinic receptors) (15). This list can be fulfilled with monepantel, an amino-acetonitrile derivative (AAD), one of the most recently developed anthelmintics with a novel mode of action based on the nematode-specific ligand-gated ion channel subunit as a potential drug target (16).

Various commercial broad-spectrum drugs have been successfully used in the control of sheep GINs over the last 50 years (17,18). However, their inappropriate use has led to the development of anthelmintic resistance (AR) in the parasites, which now occurs with virtually all commercially available drugs, especially BZ (19). In this context, high treatment frequency, inadequate anthelmintic dosing (especially underdosing) and prolonged use of the same anthelmintic class are considered the main risk factors for the development of AR (20). According to a preliminary report, the annual cost of AR in Europe is around €38 million, with a tendency to increase significantly in the future due to the increasing prevalence of resistant populations and the emergence of helminths resistant to several anthelmintic classes (13). Although monepantel has a different mode of action, resistance has already been reported (21), and the current state of the art

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suggests that AR develops in new drugs as early as 3-9 years after launch (18,22).

Other problems associated with the exclusive use of chemical drugs include the environmental consequences of anthelmintic use and public health risks. Most of these drugs are excreted in the faeces and urine, and some of them, such as ML, are not fully biotransformed in the animal organism. They also tend to accumulate in soil and groundwater and thus pose a high risk to many non-target organisms such as beneficial arthopods, including dung beetles, various flies, but also fish and other aquatic organisms (23). Residues of such pharmaceuticals can contaminate meat, milk and by-products and thus pose a serious risk to human health (23,24). For this reason, the European Community has enacted a law that requires systematic and uniform control of veterinary medicines, including anthelmintics, in food in all member countries (25). In addition, the prices of these drugs, such as albendazole and mebendazole, continue to rise despite all these negative effects (26)

MONITORING OF ANTHELMINTIC RESISTANCE

The use of various *in vitro* and *in vivo* methods for the early detection of resistance development is crucial for the definition of appropriate strategies. However, monitoring the presence of resistance in a given area and worldwide is not an easy task, considering the complex mechanisms of resistance development and certain limitations of existing methods. Currently, the most appropriate and widely used method is the faecal egg count reduction test (FECRT), an *in vivo* method based on the collection of faecal samples before and at different time points after treatment and the measurement of the reduction in the number of parasite eggs, usually expressed as eggs per gram (EPG) (27). Several techniques can be used for FECs, including McMaster ili Cornel-Wisconsin or some novel ones such as mini-FLOTAC (28) or FECPAK (29), highly sensitive and practical techniques. In contrast to the other tests, the FECRT allows the evaluation of drug efficacy for all anthelmintic classes in all animal species and for multiple parasite species without the need to sacrifice the animals. In addition, it can be performed on-site without the need for a diagnostic reference laboratory or specialised equipment and/or expertise (27). According to the instructions of the World Association for the Advancement of Veterinary Medicine (WAAVP), a reduction of GIN eggs below 95% indicates the presence of drug resistance. The results of the FECRT should be interpreted carefully when the FEC is low and the results do not always correlate with the worm burden in adult animals, although they are very reliable in young animals (27,30,31). The other *in vivo* method is the controlled efficacy test (CET), which is even more precise than the FECRT, in which the animals are artificially infected, treated and then slaughtered and the worm burden is counted. However, it is very intensive and requires the sacrifice of animals, which makes it less practical (32).

Among the *in vitro* methods, the egg hatch test (EHT) and the larval development test (LDT) are the most commonly used. The EHT is used to measure the ovicidal effect of decreasing concentrations of anthelmintics. After a 48-hour incubation of the eggs with anthelmintics at 27 °C, the remaining eggs and the hatched larvae (L_1) are counted and the inhibition of hatchability is calculated. It is one of the standardized tests (33) recommended by the WAAVP and it was developed only for the detection of AR with benzimidazoles, as they have ovicidal effects (18). However, it is the most commonly used laboratory test in practice. The only *in vitro* test that allows the detection of resistance to all groups of anthelmintics regardless of their mechanism of action is the LDT (30), which is based on the ability of larvae to survive and develop in different concentrations of anthelmintics (18). This test is more intensive that EHT and there are several ways to perform it, including the preparation of suspensions and incubation under conditions of increased humidity for 48 hours, the addition of increasing concentrations of the drug and a re-incubation of 5 days, after which the L_3 are counted to determine the survival of the larvae. The other method is a micro-agar LDT based on the preparation of drug concentrations, their dissolution in dimethyl sulfoxide and dilution in distilled water. After 7 days of incubation of the eggs, drug concentration and culture medium containing yeast extracts and salt solutions, the procedure is the same (30). Both EHT and LDT include the calculation of the IC_{50} parameter, which is used to interpret the results and for comparison.

The other *in vitro* tests used to detect AR are the larval motility test (LMT), the larval paralysis test and the adult worm motility test (AWMT), which are used less frequently than EDT and LDT but can

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confirm their results. Recently, various molecular techniques based on the detection and/or analysis of nucleic acid molecules (DNA or RNA) have also been used. These tools are able to directly measure the genetic differences between susceptible and resistant populations and can therefore also be used as a means to quantify the composition of the parasite community (34). Listed below are the most common advanced methods that are efficiently used to detect AR: Restriction fragment length polymorphisms (RFLPs), allele-specific PCR (A-SPCR), diagnostic PCR, real-time PCR, direct sequencing, pyrosequencing, deep amplicon sequencing, microsatellite markers, loop-mediated isothermal amplification (LAMP), P-glycoprotein probe, and transmembrane functional analysis (35).

METHODS FOR DELAYING ANTHELMINTIC RESISTANCE AND SUSTAINABLE CONTROL OF NEMATODES – RATIONAL USE OF ANTHELMINTICS

The methods for delaying AR refers to the strategies for sustainable control of GINs in sheep, which are described in our previous review paper (36). Here, an attempt will be made to explore these strategies further with new facts and from a slightly different aspect, especially regarding on how to reduce an application of commercial anthelmintics. As mentioned above, AR is considered an inevitable phenomenon caused by rare mutations, and since it is impossible to stop this process completely. However, there are certain strategies that can be used to delay the development of resistance and slow its spread so that the efficacy of drugs can be maintained for as long as possible. These include strategies for the rational application of commercial anthelmintics based on refugia, that refers untreated hosts or environments that allow drug-susceptible parasites to survive despite exposure to drugs (37). The best known of these strategies are targeted treatments (TT) and targeted selective treatments (TST). TT refers to treating all animals in a given herd at the most appropriate times to reduce the number of treatments in a herd and thus minimize contamination of the pasture with resistant genotypes. This can result in longer intervals between treatments while achieving the same effects (38). The TST is even more specific as it refers to the individual selection of animals within the herd for treatment and uses logical, specific criteria by which this selection is made (39). In this way, only the animals that are susceptible to GIN infection or contaminate the pasture the most are treated, which is a continuous source of worms in refugia (38). The selection criteria are not easy to determine as many different markers can be used as indicators of TST:

1) Parasitological indicators (FEC and determination of EPG)
2) Pathophysiological indicators (anaemia caused by hematophagous parasites – FAMACHA score, DAG score, DISCO score, body condition score)
3) Performance-related indicators (milk production, live weight gain) (39,40)

Each of these markers has advantages and disadvantages, and therefore their combination is most appropriate. For example, in a study by Soto-Barrientos et al. (41), the combination of FEC, FAMACHA and body condition score in sheep and goats in Mexico led to the avoidance of 70% of unnecessary treatments.

Combinations of anthelmintics with similar spectrum of activity and different mechanisms of action and resistance are widely used in several regions of the world for the control of sheep nematodes. This strategy is proposed to enable effective control of nematodes in the presence of single or multiple resistance and to slow down the development of resistance to the individual anthelmintic classes. Three main outcomes are possible when combining drugs: indifference, antagonism, additive or synergistic effects. The aim is therefore to achieve the third option, where the additive effect is given as $(A + B) = 1 - ((1 - a) \times (1 - b))$, (the efficacy of one drug is a and that of the other is b). In contrast, a synergistic effect exists when the response obtained after their combined administration is greater than additive (42). In any case, a fixed-dose combination of anthelmintics could slow down the development of AR by allowing the highest possible elimination of nematodes, with parasites surviving one agent being eliminated by the other. However, the use of anthelmintic combination products does not eliminate the significant resistance risk posed by dosing strategies that allow cattle to graze on clean (low contamination) pastures after treatment (43).

Rotation of different classes of anthelmintics approximately annually has also been widely promoted and adopted as a strategy to delay AR development in nematodes. This strategy is based on the expectation that resistant worms have lower ecological fitness than susceptible worms (at least in the early stages of selection), so that a return to

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susceptibility can be expected in years when a different class of anthelmintic is used (44). The rotation of drugs also aims to prolong the efficacy of each of them and allow susceptibility to return when they are not used. However, this only appears to be possible when AR is at an early stage of development, even before it can be detected, when natural selection can reduce the number of resistant parasites. Therefore, this strategy can be implemented in novel or future drugs, considering the level of AR development in currently used anthelmintics (45).

METHODS FOR DELAYING ANTHELMINTIC RESISTANCE AND SUSTAINABLE CONTROL OF NEMATODES – ALTERNATIVE STRATEGIES

Phytotherapy

Phytotherapy has been defined by the World Health Organization (WHO) as the medical discipline that allows the correct use, for preventive or curative purposes, of medicinal plants and their derivatives (phytotherapics or phytomedicaments), in relation to the pharmacological properties of their chemical constituents (46). In the context of the treatment of gastrointestinal nematodes in sheep, this practice is a relatively new approach on which studies have been conducted in the last 20-25 years, especially in recent years (47). Herbal anthelmintics include different types of preparations that can be used in animals in different ways. First, some reports claim that sheep infected with gastrointestinal nematodes consume more plants containing bioactive anthelmintic compounds than uninfected animals, which is known as zoopharmacognosy or self-medication (48). In these cases, animals on pasture focus on, for example, tannin-rich legumes to restore or maintain their health. It has been shown that sheep infected with *H. contortus* eat more tannin-rich *Lysiloma latisiliquum* (Tzalam) than uninfected animals (49). Villalba et al. (50) demonstrated that parasitized lambs showed a greater preference for alfalfa (*Medicago sativa*) and a mixture of 90 % alfalfa and 10 % quebracho tannin than non-parasitized animals, while these differences disappeared when the parasite load was removed by regular therapy. A better understanding of this bihevioral mechanism can help researchers develop innovative and more sustainable management strategies to improve ruminant health and welfare (48).

These findings can also be applied in the context of supplementary feeding of animals to treat or prevent diseases caused by GINs (51). Although bioactive metabolites can reduce the digestibility of food, this approach has been shown to reduce the number of nematode eggs in feces, but also the number of parasites present in general, and also negatively affect the development of some species, including *H. contortus* and *T. colubriformis* (52). This includes a large number of plant species, such as hornworts and greater trefoil (*Lotus corniculatus*, *L. pedunculatus*), sulla (*Hedysarum coronarium*) and sainfoin (*Onobrychis viciifolia*) (51). In some cases, bioactive molecules can enter the feces and interfere with the larval hatching process or even the mobility of infective larvae, which means a reduction in the potential infectivity of eggs in the feces and less contamination of the pasture by larvae (52).

Herbal products such as extracts or essential oils can also be used to treat GIN infections. Plant extracts are defined as concentrated preparations of liquid, solid or viscous consistency containing a variety of secondary metabolites in different concentration ranges (53). These include various alkaloids, glycosides, phenols, terpenoids and flavonoids. Extracts can be categorized as aqueous or alcoholic, and various methods can be used to obtain plant extracts, such as maceration, infusion, percolation and decoction, but also Soxhlet extraction, microwave or ultrasound-assisted extraction, etc. (54). On the other hand, essential oils (EOs) are highly concentrated substances extracted from different parts of plants (leaves, stems, flowers, seeds, roots, etc.) and contain a mixture of volatile compounds produced by the secondary metabolism of aromatic plants (55). Therefore, EOs are pure and highly concentrated, while extracts are diluted but still highly potent, and this is the main difference between these two plant products (56). EOs also contain a large number of bioactive components from different chemical groups, such as hydrocarbon terpenes, terpenoids (which can be various phenols, alcohols, aldehydes, ketones, oxides, esters), phenylpropanoids, etc. (57). Like extracts, EO can also be obtained by various extraction methods, including distillation (hydrodistillation and the so-called steam distillation), solvent extraction, supercritical fluid extraction, etc. However, both plant extracts and EOs have the similar problems with the variability of their chemical composition depending on the many factors such as genetic characteristics of plants, climatic and microclimatic

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factors, soil properties, the presence of some organisms and microorganisms in plant environment, the time of harvest and extraction methods, which can hinder the standardization of these products (58). Also, their active ingredients are degradable and can be inactivated to some extent in animal organism, which can affect the efficacy *in vivo*. However, there are different ways on how to overcome this problem listed in our previous review manuscripts (36,47), which includes besides all the use of encapsulation techniques to protect the active ingredints or the application of extracts or EOs with other methods in integrated approach.

The evaluation of the anthelmintic efficacy of essential oils against GINs was also the topic of our research group. In two separate studies, more than twenty EOs were tested against GINs in sheep. To evaluate their anthelmintic potential *in vitro*, an egg hatch test was carried out in which oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), winter savory (*Satureja montana*), summer savory (*Satureja hortensis*), fennel (*Foeniculum vulgare*), basil (*Ocimum basilicum*), peppermint (*Mentha x piperita*), wild mint (*Mentha spicata*) and hyssop oil showed the highest efficacy. The effect varied depending on the oil used, the chemotype of the plant and the concentrations used. At some concentrations, the effect of some of these EOs was higher than that of thiabendazole, the positive control applied at standard concentration. Five EOs were selected also for an *in vivo* study performed by using faecal egg count reduction test. Oregano and peppermint showed the strongest effects when applied intraruminally, which meant the highest field efficacy for some of the plant products at the tested dose (150 mg/kg). The chemical composition was determined for all EOs tested, and carvacrol, thymol, anethole, p-cymene, γ-terpinene, carvone, pinocamphone and linalool were identified as the bioactive compounds most responsible for the anthelmintic effect. Both laboratory and experimental tests were performed on animals with natural mixed infections, identifying the genera *Haemonchus*, *Trichostrongylus*, *Teladorsagia* and *Chabertia* in the coproculture study. Importantly, physical observation and analysis of hematological and biochemical blood parameters in the animals during the field trials did not reveal any toxic effects or side effects at the doses tested, suggesting the safety of these natural products (59-63).

Despite some limitations, the use of EO and plant extracts in the control of sheep GIN has many advantages. Firstly, their rich chemical composition can contribute to high efficacy against different parasite stages (eggs, larvae, adults), as shown in various studies so far (47,59-63). So far, they are known to have signicant negative effects on the survival, reproduction, development, behavior and metabolic pathways of nematodes (64). Since their bioactive compounds belong to different chemical groups with different mechanisms of action, the likelihood of developing AR is probably lower than with commercial drugs. Environmental and public health aspects are also in favor of herbal anthelmintics, as they are natural and biodegradable. They are also often considered safer for hosts than synthetic chemical compounds. Finally, the large number of plant species offers the possibility to find the most suitable ones, also from a financial point of view, especially in countries with a developed biodiversity such as Serbia (47,59-63).

Biological methods

Biological control (or biocontrol) is defined as any activity of one species that reduces the adverse effect of another (64). In the context of sheep GINs, this refers to the use of their natural enemies to reduce the number of free-living stages on pasture. Biocontrol can be direct, when the other species have a direct effect on the GINs, or indirect, when they destroy the habitat of parasites (12). Several organisms can be used for direct biocontrol of GINs. The most studied are nematophagous fungi, carnivorous fungal species found worldwide. Their mechanisms of interaction with nematodes can be divided into four groups:

1) nematode-feeding fungi that use sticky traps (nets),
2) endoparasitic fungi,
3) opportunistic fungi that colonise nematode eggs, cysts and females (this applies above all to plant-parasitic nematodes), and
4) toxin-producing fungi (65)

The first two groups are considered to have the greatest potential, especially the nematode-feeding fungi (genera *Duddingtonia*, *Arthrobotrys* and *Monacrosporium*). They can be used to reduce the number of infective GINs larvae on pasture (12). In most studies, these fungi are administered via the feed in the form of sodium alginate pellets. Since the spores only activate and attack the GIN larvae after they have passed through the gastrointestinal tract and are excreted in the faeces, they have no side effects for the hosts (66). Among these fungi, *D.*

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flagrans shows the highest potential, achieving an effect of 37.6–91.5 % on sheep GINs (12).
Other organisms that can be used for direct control are *Bacillus thuringiensis*, which produces δ-endotoxin (crystal protein) that shows high efficacy *in vitro* against adult *H. contortus*, *T. colubriformis* and *O. circumcinta*. Various viruses, the soil amoeba *Theratromyxa weberi* and protozoa are also mentioned (67). Free-living nematodes such as *Butlerius butleri* and the mite *Lasioseius penicilliger* have also shown potential for biocontrol of GINs, but may pose a risk to non-target organisms as they are not selective (12). Dung beetles and earthworms are most often mentioned in the context of indirect control, as they feed on and decompose faeces that contain GIN larvae. During feeding, both can consume nematodes in the soil or faeces or actively participate in the destruction of eggs and larvae. They can also transport them to deeper soil layers, where it is practically impossible for the larvae to reach the surface as infective larvae (67).

Vaccine development

Vaccination is considered a sustainable intervention strategy to control GIN infections in livestock, including sheep (68). They are considered environmentally friendly and, similar to phytotherapy, are likely to be less susceptible to the development of AR (69). Currently, progress has been made in researching vaccines against GINs in many economically important species such as *H. contortus*, *O. ostertagi*, *T. circumcincta*, *C. oncophora* and *T. colubriformis* (68). In general, these vaccines can be classified as follows:
1) Hidden antigens, which are generally found in the intestines of the parasites and are not recognized by the sheep's immune system
2) Natural antigens that are expressed during infection and recognized by the sheep (70)
The list of potential antigens is long and includes H11, H-gal-GP, ES15/24, thiol-binding fraction (ES), AC-5 (ES), rPEP1, rMEP1, rMEP3, rMEP4, rHc23, rES15/24, TciAPY-1 + TciMEP-1, mTciAPY-1 + TciMEP-1, somatic antigen, etc. These antigens were isolated from adult GINs and different larval stages ($L_{3\text{-}5}$), but also from other nematodes (*Caenorhabditis elegans*), bacteria (*E. coli*, *P. pastoris*) or insect cells. The vaccines also contain various adjuvants and are administered i.m., s.c. or intrarectally at different intervals depending on the vaccination schedule. However, only one vaccine called "Barbervax" (https://barbervax.com/) against one of the GIN species (*H. contortus*) in sheep has been commercialized so far, which is based on natural antigens derived from the glycoproteins of the intestine of adult worms (68). This vaccine is available in Australia, the UK and South Africa and showed an effect on FECRT of more than 80 % with a high antibody titer in two different feeding regimens (71). Vaccines investigated so far were only directed against one GIN species, which is a problem as mixed infections occur in most practical cases. The main problem is the genetic variations and immunoregulatory properties of gastrointestinal nematodes, which complicate the development of vaccines (72). However, with the development of bioinformatics and methods such as reverse vaccinology, the immunoprophylactic and immunotherapeutic spectrum to combat these parasitic pathogens is expected to expand (70).

Genetic selection

One of the methods to mitigate the effects of GINs in sheep is also to select animals that are naturally resistant to these infections in order to increase host resistance. This is only possible if the desired trait must have non-zero heritability (a trait with $h^2 = 0$ has no additive genetic variance and a trait with $h^2 = 1$ is completely determined by additive genetic effects). In this context, breeding selection is based on two traits: resistance, which refers to the ability of an animal to reduce parasite load, and resilience, which represents the ability of an animal to maintain its performance in the face of infection. Similar to the TST, various markers can be used to measure resistance (73). For the FEC, various qualitative reviews show that the heritability of resistance in animals ranges from 0 to 0.65 (74), with typical values of 0.2 to 0.4 (75). For FAMACHA scores and nematode-specific antibody responses, the heritability is 0-0.4 (76,77). Since higher resistance is associated with higher animal production, breeding for higher resistance should also lead to better performance. For example, some studies have already shown that genetic selection has reduced FEC by almost 70 % (78). However, these measures are difficult to implement on small production farms, and technical and infrastructural issues should also be considered in genetic selection programs, which are a particular problem in less developed countries (Zvinorova et al., 2016).

Other methods

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Pasture provides the environment for parasite eggs and larvae. Therefore, certain pasture management practices that attempt to keep the pasture clean result in lower worm infestations (79). The aim of these strategies is to provide low-risk pastures (with low larvae numbers) for the most susceptible animals (weaning and lactating ewes, young animals). The aim is also to avoid exposure that would lead to clinical disease and loss of production, while allowing sheep to build up immunity to parasites (45,79). Three main strategies are proposed to reduce pasture contamination: a preventive strategy, in which animals are prevented from contaminating pasture by moving clean animals to clean pastures; an avoidance strategy, in which moderate existing infection is eliminated by anthelmintic treatment and movement of treated animals to clean pastures; and a dilution strategy, in which resistant adult animals are grazed together with susceptible animals to dilute their parasite egg-enriched feces. Based on these strategies, the following grazing management practices are proposed (79):

1) Pasture rotation system – this involves subdividing the pasture where each part is grazed for a short period and then rested for a longer period to induce death of larvas;
2) Combined cropping/livestock system – larvae numbers are reduced over time by resting the pasture for a period of time while hay/silage is grown and harvested;
3) Alternate grazing system - grazing different species, e.g. sheep with cows, as they do not share common parasite species;
4) Dose & Move – moving animals from pasture to pasture after deworming;
5) Stocking density – lowering the stocking density, as a high number of animals increases the larval load
6) Grass height – as the majority of larvae live in the first 5 cm of vegetation, the animals should be grazed on longer (10 cm) vegetation;
7) Grazing time – limit the grazing time in strong sunlight, as the larvae move to the top of the grass when light intensity is low;
8) Zero grazing – practiced for fat lamb production, where animals are kept in a dry barn without grass.

In addition to these strategies, supplementary feeding could be used to increase the resistance of sheep to GINs. Indeed, a balanced diet provides an adequate source of nutrients and an acceptable parasite load that allows for optimal productivity levels. This refers to various vitamins (especially A, D and B-complex), minerals (zinc, iron, cobalt, sodium, potassium and phosphorus) and proteins, which are crucial for the development of functional immunity against parasites. In this way, a reduction in the pathophysiological effects of parasite infestation and improved productivity could be achieved while reducing the use of anthelmintics (52). Copper oxide wire particles (COWP) have also shown promising anthelmintic activity against GINs in small ruminants when administered as an oral bolus. Although the exact mechanism of action is unknown, it is known that COWP reduces both fertility and the number of *H. contortus* worms in the abomasum. Importantly, oral administration of COWP has also been shown to be a safe and effective method (80).

Combination of different strategies – *integrated approach*

Since all of the above strategies have their advantages, but also limitations and the exclusive use of commercial drugs is no longer sustainable, many researchers agree that a combination of different methods should be performed in GINs management programs. Firstly, the alternatives mentioned can enhance the effect of the regular drugs. For example, it has been shown that the combination of carvacrol in conjunction with nicotinic acetylcholine receptor agonists such as imidazothiazole or GABA receptor agonists such as avermectins and piperazine could have a synergistic effect against various nematodes (81-82). Specifically, *Nigella* sativa oil has been shown to enhance the effect of ivermectin against *H. contortus*, but also against some other parasites such as *Moniezia expansa* and *Fasciola gigantica*, compared to independent use (83). In a study by Vilela et al. (84), the combination of *D. flagrans*-based pellets and levamisole hydrochloride (5%) was shown to have a synergistic effect, as the group of animals receiving both treatments had lower EPG levels compared to the isolated application of the drug. Copper chloride and copper sulphate in combination with nitroxynil showed up to 52% increase in efficacy beyond the expected additive results, indicating a synergistic/drug enhancing interaction (85).

In some studies, different alternatives in combination showed high anthelmintic activity. For example, Bombou et al. (86) investigated the combination of mixed grazing and dietary supplementation to improve the protective response of the host and the production performance of goats.

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Both strategies had a positive effect - FEC was significantly lower in the mixed grazing groups, while PCV, body condition score and live weight were significantly higher in the supplemented animals, suggesting that this combination can improve animal productivity. In a study by Werne et al. (87), the synergistic effect of combining a relatively resistant breed and a diet rich in condensed tannins against sheep GINs could not be clearly demonstrated, but a proportion of only 55% sainfoin in the diet resulted in a lower FEC compared to the control group. The combination of the ethanol extract of *Ananas comosus*, drenched weekly for 6 weeks using a stomach tube (100 mg/kg), with 1 g of the fungal product *Clonostachys rosea* given daily, effectively reduced faecal egg and larva counts, larval development and the number of infectious larvae (L_3) in the pasture (88). It can be concluded that an integrated approach, based on the rational use of anthelmintics and the use of alternative strategies, should form the basis for the future treatment of GIN infections in order to reduce the use of commercial drugs, combat anthelmintic resistance and achieve sustainable control.

LITERATURE

1. *López-Rodríguez G., Zaragoza-Bastida A., Olmedo-Juárez A., Rosenfeld M.C., Rivero-Perez N. Gastrointestinal nematodes in sheep and their anthelmintic resistance. A subject under discussion in Mexico. Journal of the Selva Andina Animal Science 2023, 10(2):116-129.*
2. *Waller P.J. Sustainable nematode parasite control strategies for ruminant livestock by grazing management and biological control. Animal Feed Science and Technology 2006, 126(3-4):277-89.*
3. *Kulišić Z., Aleksić N., Đorđević M., Gajić B., Tambur Z., Stevanović J., Stanimirović Z. Prevalence and intensity of infection with gastroitnestinal nematodes in eastern Serbia. Acta Veterinaria (Beograd) 2013, 63(4): 429-436.*
4. *Pavlović I., Becskei Z., Ivanović S., Petrović M.P., Savić M., Petrović V.C., Bojkovski J.A. Biodiversity of helminths of sheep breed in Vojvodina (northern Serbia). Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Agriculture 2017, 74(2):162-166.*
5. *Borkowski E.A., Avula J., Redman E.M., Sears W., Lillie B.N., Karrow N.A., Menzies P.I., Gilleard J.S., Peregrine A.S. Correlation of subclinical gastrointestinal nematode parasitism with growth and reproductive performance in ewe lambs in Ontario. Preventive Veterinary Medicine 2020, 185:105175*
6. *Charlier J., Rinaldi L., Musella V., Ploeger H.W., Chartier C., Rose Vineer H., Hinney B., von Samson-Himmelstjerna G., Băcescu B., Mickiewicz M., Mateus T.L., Martinez-Valladares M., Quealy S., Azaizeh H., Sekovska B., Akkari H., Petkevicius S., Hektoen L., Höglund J., Morgan E.R., Bartley D.J., Claerebout E. Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe. Preventive Veterinary Medicine 2020, 182:105103*
7. *Sheferaw D., Mohammed A., Degefu A. Distribution and prevalence of gastrointestinal tract nematodes of sheep at highland and midland areas, Ethiopia. Journal of Parasitic Diseases 2021, 45(4):995–1001.*
8. *Tachack E.B., Oviedo-Socarrás T., Pastrana M.O., Pérez-Cogollo L.C., Benavides Y.H., Pinto C.R., Garay O.V. Status of gastrointestinal nematode infections and associated epidemiological factors in sheep from Córdoba, Colombia. Tropical Animal Health and Production 2022, 54(3):171.*
9. *Giovanelli F., Mattellini M., Fichi G., Flamini G., Perrucci S. In vitro anthelmintic activity of four plant-derived compounds against sheep gastrointestinal nematodes. Veterinary Sciences 2018, 5(3):78.*
10. *Beleckė A., Kupčinskas T., Stadalienė I, Höglund J, Thamsborg S.M., Stuen S, Petkevičius S. Anthelmintic resistance in small ruminants in the Nordic-Baltic region. Acta Veterinaria Scandinavica 2021, 63:18.*
11. *Bosco A., Kießler J., Amadesi A., Varady M., Hinney B., Ianniello D., Paola Maurelli M., Cringoli G., Rinaldi L. The threat of reduced efficacy of anthelmintics against gastrointestinal nematodes in sheep from an area considered anthelmintic resistance-free. Parasites & Vectors 2020, 13:457.*
12. *Szewc M., De Waal T., Zintl A. Biological methods for the control of gastrointestinal nematodes. The Veterinary Journal 2021, 268:105602.*
13. *Rose Vineer H., Morgan E.R., Hertzberg H., Bartley D.J., Bosco A., Charlier J., Chartier C., Claerebout E., de Waal T., Hendrickx G., Hinney B., Höglund J., Ježek J., Kašný M., Keane O.M., Martínez-Valladares M., Mateus T.L., McIntyre J., Mickiewicz M., Munoz A.M., Phythian C.J., Ploeger H.W., Rataj A.V., Skuce P.J., Simin S., Sotiraki S., Spinu M., Stuen S., Thamsborg S.M., Vadlejch J., Varady M., von Samson-Himmelstjerna G., Rinaldi L. Increasing importance of anthelmintic resistance in European livestock: creation and meta-analysis of an open database. Parasite 2020, 27:69.*
14. *Castagna F., Palma E., Cringoli C., Bosco A., Nisticò N., Caligiuri G., Britti D., Musella V. Use of complementary natural feed for gastrointestinal nematodes control in sheep: Effectiveness and benefits for animals. Animals 2019, 9(12):1037.*

15. *Solomon L., Haile G., Ahmed N.A., Abdeta D., Galalcha W., Hailu Y. Epidemiology and field efficacyof anthelmintic drugs associatedwith gastrointestinal nematodesof sheep in Nejo district, Oromia, Ethiopia. Scientific Reports 2024, 14:6841.*
16. *Canton C., Canton L., Lifschitz A., Dominguez M.P., Alvarez L., Ceballos L., Mate L., Lanusse C., Ballent M. Monepantel-based anthelmintic combinations to optimize parasite control in cattle. International Journal for Parasitology 2023, 53(8):441-449.*
17. *Kaplan R. Biology, epidemiology, diagnosis, and management of anthelmintic resistance in gastrointestinal nematodes of livestock. Veterinary Clinics of North America: Food Animal Practice 2020, 36(1):17-30.*
18. *Fissiha W., Kinde M.Z. Anthelmintic resistance and its mechanism: A review. Infection and Drug Resistance 2021, 14:5403-10.*
19. *Dolinská M., Ivanišinová O., Königová A., Várady M. Anthelmintic resistance in sheep gastrointestinal nematodes in Slovakia detected by in-vitro methods. BMC Veterinary Research 2014, 10:233.*
20. *Alaro T., Dulo F., Wodajo W., Mathewos L. Anthelmintic resistance of gastrointestinal nematodes of communally-grazing goats in Humbo district, Southern Ethiopia. Veterinary Medicine: Research and Reports 2023, 14:185-194.*
21. *Mederos A.E., Ramos Z., Banchero G.E. First report of monepantel Haemonchus contortus resistance on sheep farms in Uruguay. Parasites & Vectors 2014, 7:598.*
22. *Jayawardene K.L.T.D., Palombo E.A., Boag P.R. Natural products are a promising source for anthelmintic drug discovery. Biomolecules 2021, 11(10):1457.*
23. *Reyes-Guerrero D.E., Olmedo-Juárez A., Mendoza-de Gives P. Control and prevention of nematodiasis in small ruminants: background, challenges and outlook in Mexico. Revista mexicana de ciencias pecuarias 2022, 12(Supl 3):186-204.*
24. *Castagna F., Bava R., Musolino V., Piras C., Cardamone A., Carresi C., Lupia C., Bosco A., Rinaldi L., Cringoli G., Palma E., Musella V., Britti D. Potential new Therapeutic approaches based on Punica granatum fruits comparedto synthetic anthelmintics for the sustainable control of gastrointestinal nematodes in sheep. Animals, 2022, 12:2883.*
25. *Jedziniak P., Olejnik M., Rola J.G., Szprengier-Juszkiewicz T. Anthelmintic residues in goat and sheep dairy products. Bulletin of the Veterinary Institute in Pulawy 2015, 59:515-518.*
26. *Fleit M., Vokman H., Alpern J.D., Lindrose A.R., Stauffer W., Mitre E. Impact of Anthelmintic Price Increases on Practice Patterns of Healthcare Providers Caring for Immigrant and Refugee Populations in the United States. The American Journal of Tropical Medicine and Hygiene 2021, 104(2):718-723.*
27. *Kaplan R.M., Denwood M.J., Nielsen M.K., Thamsborg S.M., Torgerson P.R., Gilleard J.S., Dobson R.J., Vercruysse J., Levecke B. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guideline for diagnosing anthelmintic resistance using the faecal egg count reduction test in ruminants, horses and swine. Veterinary Parasitology 2023, 318:109936*
28. *Cringoli G., Maurelli M.P., Levecke B., Bosco A., Vercruysse J., Utzinger J., Rinaldi L. 2017. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nature Protocols 2017, 12(9):1723-1732.*
29. *Rashid M.H., Stevenson M.A., Waenga S., Mirams G., Campbell A.J.D., Vaughan J.L., Jabbar A. Comparison of McMaster and FECPAKG2 methods for counting nematode eggs in the faeces of alpacas. Parasites & Vectors 2018, 11(1):278.*
30. *Mphahlele M., Molefe N., Tsotetsi-Khambule A., Oriel T. Anthelmintic resistance in livestock, In Helminthiasis. Ed. O.O. Okwa. IntechOpen, London, 2019.*
31. *Sabatini G.A., Borges F.D.A., Clarebout E., Gianechini L.C., Höglund J., Kaplan R.W., Lopes W.D.Z., Mitchell S., Rinaldi L., von Samson-Himmelstjerna G., Steffan P., Woodgate R. Practical guide to the diagnostics of ruminant gastrointestinal nematodes, liver fluke and lungworm infection: interpretation and usability of results. Parasites & Vectors 2023, 16:58.*
32. *Love J.W., Kelly L.A., Lester H.E., Nanjiani I., Taylor M.A., Robertson C. Investigating anthelmintic efficacy against gastrointestinal nematodes in cattle by considering appropriate probability distributions for faecal egg count data. International Journal for Parasitology: Drugs and Drug Resistance 2017, 7:71-82.*
33. *von Samson-Himmelstjerna G., Coles G.C., Jackson F., Bauer C., Borgsteede F., Cirak V.Y., Demeler J. Donnan A., Dorny P., Epe C., Harder A., Höglund J., Kaminsky R., Kerboeuf D., Küttler U., Papadopoulos E., Posedi J., Small J., Várady M., Vercruysse J., Wirtherle N. Standardization of the egg hatch test for the detection of benzimidazole resistance in parasitic nematodes. Parasitology Research 2009, 105(3): 825-834.*
34. *Kotze A.C., Gilleard J.S., Doyle S., Prichard R.K. Challenges and opportunities for the adoption of molecular diagnostics for anthelmintic resistance. International Journal for Parasitology: Drugs and Drug Resistance 2020, 14:264-273*

35. *Hassan N.M.F., Ghazy A.A. Advances in diagnosis and control of anthelmintic resistant gastrointestinal helminths infecting ruminants. Journal of Parasitic Diseases 2022, 46(3):901-915.*
36. *Štrbac F., Krnjajić S., Stojanović D., Ratajac R. Novel strategies for the control of gastrointestinal nematodes in sheep. Veterinary Journal of Republic of Srpska (Banja Luka) 2023, XXIII(1-2):227-245.*
37. *Hodgkinson J.E., Kaplan R.M., Kenyon F., Morgan E.R., Park A.W., Paterson S., Babayan S.A. Refugia and anthelmintic resistance: concepts and challenges. International Journal for Parasitology: Drugs and Drug Resistance 2019, 10:51-57*
38. *Kenyon F., Greer A.W., Coles G.C., Cringoli G., Papadopoulus E., Cabaret, J., Berrag B., Varady M., Van Wyk J.A., Thomas E., Vercruysse J., Jackson F. The role of target selective treatments in the development of refugia-based approaches to the control of gastrointestinal nematodes of small ruminants. Veterinary Parasitology 2009, 164(1): 3–11.*
39. *Edith R, Harikrishnan TJ, Balagangatharathilagar M. Targeted selective treatment (TST): A promising approach to combat anthelmintic resistance in farm animals. Journal of Entomology and Zoology Studies 2018 6(1):844-847.*
40. *Maurizio A., Perrucci S., Tamponi C., Scala A., Cassini R., Rinaldi L., Bosco A. Control of gastrointestinal helminths in small ruminants to prevent anthelmintic resistance: the Italian experience. Parasitology 2023 150(12):1105–1118.*
41. *Soto-Barrientos N., Chan-Pérez I.I., España-España E., Novelo-Chi L.K., Palma-Ávila I., Ceballos-Mendoza A.C., Sarabia-Hernández J.A., Santos-Ricalde R.H., Cámara-Sarmiento R., Torres-Acosta J.F.J. Comparing body condition score and FAMACHA© to identify hair-sheep ewes with high faecal egg counts of gastrointestinal nematodes in farms under hot tropical conditions. Small Ruminant Research 2018, 167:92-99.*
42. *Bartram D.J., Leathwick D.M., Taylor M.A., Geurden T., Maeder S.J. The role of combination anthelmintic formulations in the sustainable control of sheep nematodes. Veterinary Parasitology 2012, 186(3–4):151-158*
43. *Geary T., Hosking B., Skuce P.J., von Samson-Himmelstjerna G., Maeder S., Holdsworth P., Pomroy W., Vercruysse J. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) Guideline: Anthelmintic combination products targeting nematode infections of ruminants and horses. Veterinary Parasitology 2012, 190(s1-2):306-316*
44. *Leathwick D.M. Managing anthelmintic resistance – Parasite fitness, drug use strategy and the potential for reversion towards susceptibility. Veterinary Parasitology 2012, 198(1–2):145-153*
45. *Abbott K.A., Taylor M., Stubbings L.A. Sustainable worm control strategies for sheep,4th Ed., A Tehnical Manual for Veterinary Surgeons and Advisers, SCOPS, UK, 2017; pp: 40.*
46. *Allegra S., De Francia S., Turco F., Bertaggia I., Chiara F., Armando T., Storto S., Mussa M.V. Phytotherapy and drugs: Can their interactions increase side effects in cancer patients? Journal of Xenobiotics 2023, 13(1):75–89.*
47. *Štrbac F., Bosco A., Pušić I., Stojanović D., Simin N., Cringoli G., Rinaldi L., Ratajac R. The use of essential oils against sheep gastrointestinal nematodes. In Animal Health Perspectives, Volume I. Ed. R.Z. Abbas, A. Khan, P. Liu, M.K. Saleemi, M.K. Unique Scientic Publishers, Faisalabad, Pakistan, 2022, pp 86–94.*
48. *Villalba J.J., Miller J., Ungar E.D., Landau S.Y., Glendinning J. Ruminant self-medication against gastrointestinal nematodes: evidence, mechanism, and origins. Parasite 2014, 21:31.*
49. *Lisonbee L.D., Villalba J.J., Provenza F.D., Hall J.O. Tannins and self-medication: Implications for sustainable parasite control in herbivores. Behavioural Processes 2009, 82:184–189.*
50. *Villalba J.J., Provenza F.D., Rogosic J. Preference for flavored wheat straw by lambs conditioned with intraruminal infusions of starch administered at different times after straw ingestion. Journal of Animal Science 1999, 77:3185–3190.*
51. *Hoste H., Torres-Acosta J.F.J., Sandoval-Castro C.A., Mueller-Harvey I., Sotiraki S., Louvandini H., Thamsborg S.M., Terrill T.H. Tannin containing legumes as a model for nutraceuticals against digestive parasites in livestock. Veterinary Parasitology 2015, 212(1–2):15-17.*
52. *Torres-Acosta J.F.J., Sandoval-Castro C.A., Hoste H., Aguilar-Caballero A.J., Cámara-Sarmiento R., Alonso-Díaz M.A. Nutritional manipulation of sheep and goats for the control of gastrointestinal nematodes under hot humid and subhumid tropical conditions. Small Ruminant Research 2012, 103(1): 28-40.*
53. *Borges D.G.L., Borges F.A. Plants and their medicinal potential for controlling gastrointestinal nematodes in ruminants. Nematoda 2016, 3: e92016.*
54. *Dekebo A. Introductory Chapter: Plant Extracts. In Plant Extracts. Ed. A. Dekebo. Intechopen, London, UK, 2019.*
55. *Cimino C, Maurel OM, Musumeci T, Bonaccorso A, Drago F, Souto EMB, Pignatello R., Carbone C. Essential oils: Pharmaceutical applications and encapsulation strategies intolipid-based delivery systems. Pharmaceutics 2021, 13:327.*

56. *Bolouri P., Salami R., Kouhi S., Kordi M., Lajayer B.A., Hadian J., Astatkie T. Applications of essential oils and plant extracts in different industries. Molecules 2022, 27(24): 8999.*
57. *Morsy N.F.S. Chemical structure, quality indices and bioactivity of essential oil constituents. In Active ingredients from aromatic and medicinal plants. Ed. H.El-Shemy. Intechopen, London, UK, 2017.*
58. *Fokou J.B.H., Dongmo P.M.J., Boyom F.F. Essential oil's chemical composition and pharmacological properties. Essential oils - oils of nature. Ed. H.El-Shemy. Intechopen, London, UK, 2020.*
59. *Štrbac F. Anthelmintic efficacy of selected essential oils against gastrointestinal nematodes of sheep. PhD thesis, Faculty of Agriculture, University of Novi Sad, 2022.*
60. *Štrbac F., Bosco A., Maurelli M.P., Ratajac R., Stojanović D., Simin N., Orčić D., Pušić I., Krnjajić S., Sotiraki S., Saralli G., Cringoli G., Rinaldi L. Anthelmintic properties of essential oils to control gastrointestinal nematodes insheep—In vitro and in vivo studies. Veterinary Sciences 2022, 9:93.*
61. *Štrbac F., Krnjajić S., Maurelli M.P., Stojanović D., Simin N., Orčić D., Ratajac R., Petrović K., Knežević G., Cringoli G., Rinaldi L., Bosco A. 2023. A potential anthelmintic phytopharmacological source of Origanum vulgare (L.) essential oil against gastrointestinal nematodes of sheep. Animals 2023, 13:45.*
62. *Štrbac F., Krnjajić S., Stojanović D., Ratajac R., Simin N., Orčić D., Rinaldi L., Ciccone E., Maurelli M.P.M., Cringoli G., Bosco A. In vitro and in vivo anthelmintic efficacy of peppermint (Mentha x piperita L.) essential oil against gastrointestinal nematodes of sheep. Frontiers in Veterinary Science 2023, 10:1232570.*
63. *Piao X, Sun M, Fengping Y. Evaluation of nematocidal action against Caenorhabditis elegans of essential oil of flesh fingered citron and its mechanism. Journal of Chemistry 2020, 2020:1740938.*
64. *Kwenti T.E. Biological control of parasites. In Natural Remedies in the Fight Against Parasites Ed. H.Khater, M.Govindarajan, Giovanni Benelli. Intechopen, London, UK, 2017.*
65. *Jiang X., Xiang M., Liu X. Nematode-trapping fungi. Microbiology Spectrum 2016, 5(1):FUNK-0022-2016*
66. *Burke J.M., Miller J.E. Sustainable approaches to parasite control in ruminant livestock. Veterinary Clinics of North America: Food Animal Practice 2020, 36:89-107.*
67. *Worku E., Kiros A., Asgedom H., Tadesse B. Alternative control methods of gastrointestinal nematode infections in small ruminants: biological method and use of medicinal plant extracts. ARC Journal of Animal and Veterinary Sciences 2017, 3(2): 11-27.*
68. *Liu H., Zhang Y., Liu F., Ye L., Liu X., Wang C., Hu M. Progress and challenges for developing vaccines against gastrointestinal nematodes of ruminants. Veterinary Vaccine 2023, 2(3–4):100041*
69. *Ehsan M., Hu R.S., Liang Q.L., Hou J.L., Song X., Yan R., Zhu X.Q., Li X. Advances in the development of anti-Haemonchus contortus vaccines: challenges, opportunities, and perspectives. Vaccines (Basel) 2020, 8(3):555.*
70. *Vargas L.M., Prieto L.D., Baquero M.M., Corredor W., Alcantara-Neves N.M., Jaramillo-Hernández D.A. Vaccines for gastrointestinal parasites, a pillar of preventive medicine in veterinary practice: Systematic review. Revista de Investigación Agraria y Ambiental 2022, 13(1), 221 – 251.*
71. *Bassetto C., Almeida F., Newlands G. F. J., Smith W., Castilhos A., Fernandes S., Siqueira E. Trials with the Haemonchus vaccine, Barbervax®, in ewes and lambs in a tropical environment: Nutrient supplementation improves protection in periparturient ewes. Veterinary Parasitology 2018 2018, 264:52-57.*
72. *Britton C., Emery D. L., McNeilly T. N., Nisbet A. J., Stear M. J. The potential for vaccines against scour worms of small ruminants. International Journal for Parasitology 2020, 50(8):533-553.*
73. *Hayward A.D. Genetic parameters for resistance to gastrointestinal nematodes in sheep: a meta-analysis. International Journal for Parasitology 2022, 52(13–14):843-853*
74. *Zvinorova P.I., Halimani T.E., Muchadeyi F.C., Matika O., Riggio V., Dzama K. Breeding for resistance to gastrointestinal nematodes – the potential in low-input/output small ruminant production systems. Veterinary Parasitology 2016, 225:19-28*
75. *Bishop S.C. Possibilities to breed for resistance to nematode parasite infections in small ruminants in tropical production systems. Animal 2012, 6:741-747*
76. *Davies G., Stear M.J., Bishop S.C. Genetic relationships between indicator traits and nematode parasite infection levels in 6-month-old lambs. Animal Science 2005, 80:143-150*
77. *Shyman M.A., Fisher A.D. Genetic parameters for traits associated with resistance to Haemonchus contortus in a South African Dohne Merino sheep flock. Small Ruminant Research 2019, 176:76-88.*
78. *Eady S.J., Woolaston R.R., Barger I.A. Comparison of genetic and non-genetic strategies for control of gastrointestinal nematodes of sheep. Livestock Production Science 2003, 81(1):11-23.*
79. *Bukhari S., Sanyal P.K. Epidemiological intelligence for grazing management in strategic control of parasitic gastroenteritis in small ruminants in India – A review. Veterinary World 2011, 4(2):92-96.*
80. *Needleman A.L., Wright M.C., Schaefer J.J., Videla R., Lear A.S. Copper oxide wire particles effective against gastrointestinal nematodes in adult alpacas during a randomized clinical trial. American Journal of Veterinary Research 2022, 83(11):1-5.*

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81. *Trailović S.M., Marjanović Đ.M., Trailović J.N., Robertson A.P., Martin R.J. Interaction of carvacrol with the Ascaris suum nicotinic acetylcholine receptors and gamma-aminobutyric acid receptors, potential mechanism of antinematodal action. Parasitology Research 2015, 114:3059-3068.*
82. *Marjanović Đ. M., Zdravković N., Milovanović M., Trailović J. N., Robertson A. P., Todorović Z., Trailović S. M. Carvacrol acts as a potent selective antagonist of different types of nicotinic acetylcholine receptors and enhances the effect of monepantel in the parasitic nematode Ascaris suum. Veterinary Parasitology 2020, 278:109031.*
83. *Shalaby H.A., Abu El Ezz N.M.T., Farag T.K., Abou-Zeina H.A.A. In vitro efficacy of a combination of ivermectin and Nigella sativa oil against helminth parasites. Global Veterinara 2012, 9:465-473.*
84. *Vilela V.L.R., Feitosa T.F., Braga F.R., Vieira V.D., de Lucena S.C., de Araujo J.V. Control of sheep gastrointestinal nematodes using the combination of Duddingtonia flagransand Levamisole Hydrochloride 5%. Revista Brasileira de Parasitologia Veterinaria 2018, 27(1):27-32.*
85. *Dolenga C.J.R., dos Anjos A., Arruda E.J., Molento M.B. Copper chloride and copper sulphate in combination with nitroxynil against gastrointestinal nematodes of ruminants: A possible hitchhiking synergic effect at low concentrations. International Journal for Parasitology 2023, 53(3):177-183.*
86. *Bambou J.C., Cei W., Arquet R., Calif V., Bocage B., Mandonnet N., Alexandre G. Mixed grazing and dietary supplementation improve the response to gastrointestinal nematode parasitism and production performances of goats. Frontiers in Veterinary Sciences 2021, 8:628686.*
87. *Werne S., Isensee A., Maurer V., Perler E., Drewek A., Heckendorn F. Integrated control of gastrointestinal nematodes in lambs using a bioactive feed × breed approach. Veterinary Parasitology 2013, 198(3-4):298-304.*
88. *Ahmed M., Laing M.D., Nsahlai I.V. A new control strategy for nematodes of sheep using chlamydospores of a fungus, Clonostachys rosea f. rosea, and an ethanolic extract of a plant, Ananas comosus. Biocontrol Science and Technology 2014, 24:860-871.*

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ZNAČAJ INOVATIVNE smaXtec TEHNOLOGIJE ZA POBOLJŠANJE REPRODUKTIVNE EFIKASNOSTI MUZNIH KRAVA
THE IMPORTANCE OF THE INNOVATIVE smaXtec TECHNOLOGY FOR IMPROVING THE REPRODUCTIVE EFFICIENCY OF DAIRY COWS

Srđan Todorović[a]*, Zoran Ružić[a], Ivica Jožef[b], Ivan Galić[a]

[a]Univerzitet u Novom Sadu, Poljoprivredni fakultet, Departman za veterinarsku medicinu, Novi Sad, Srbija;
[b]Nacionalni istraživačko - obrazovni centar inovacionih tehnologija u poljoprivredi AGRO CAMPUS, Temerin, Srbija
*Corresponding author: srdjan.todorovic28@gmail.com

Z.R. https://orcid.org/0000-0001-7754-7264, I.G. https://orcid.org/0000-0002-6281-0776

SAŽETAK

U radu su prikazani rezultati istraživanja uticaja digitalnog sistema za monitoring muznih krava koji se zasniva na permanentnom praćenju tri fiziološka parametra (pomoću bolusa sa senzorima) na osnovu čijih promena se može vršiti i dijagnostika početka estrusa. Ogled je izvršen s ciljem ispitivanja uspeha ove tehnologije u pobošanju reproduktivne efikasnosti zapata. Ispitivanje je izvršeno na jednoj farmi na ukupno 50 oteljenih krava simentalske rase, optimalne telesne kondicije, podeljenih u dve grupe. U ovoj longitudinalnoj studiji praćene su životinje pre korišćenja digitalnog sistema i nakon toga. U kontrolnoj grupi je bilo 25 osjemenjenih krava na osnovu uočenih simptoma estrusa od strane osoblja na farmi dok je oglednu grupu činil 25 krava kojima je aplikovan bolus i osjemenjavanje je vršeno nakon otkrivanja estrusa koji je dijagnostikovao digitalni sistem. Rezultati ovog istraživanja pokazuju da je vrednost koncepcije kod krava iz ogledne grupe iznosila 61,54 % (16/26). Kod krava iz kontrolne grupe vrednsot koncepcije je iznosila 48,00 % (12/25). Rezultati ovog rada ukazuju da ispitivana digitalna tehnologija može značajno poboljšati vrednost koncepcije osemenjenih krava i time dati doprinos poboljšanju reproduktivne efikasnosti zapata.

Ključe reči: reprodukcija, digitalizacija, estrus, koncepcija, krave

ABSTRACT

This paper presents the results of research into the impact of a digital system for monitoring dairy cows, which is based on the permanent monitoring of three physiological parameters (using a bolus with sensors), based on which changes can be used to diagnose the onset of estrus. The experiment was carried out with the aim of examining the success of this technology in improving the reproductive efficiency of the spawn. The test was carried out on one farm on a total of 50 calving cows of the Simmental breed, optimal body condition, divided into two groups. In this longitudinal study, animals were monitored before and after using the digital system. In the control group there were 25 cows inseminated based on the observed symptoms of estrus by the farm staff, while the experimental group consisted of 25 cows that were bolus applied and insemination was performed after detection of estrus diagnosed by the digital system. The results of this research show that the value of conception in cows from the experimental group was 61.54% (16/26). In cows from the control group, the conception rate was 48.00% (12/25). The results of this work indicate that the examined digital technology can significantly improve the conception value of inseminated cows and thus contribute to improving the reproductive efficiency of the herd.

Keywords: reproduction, digitalization, estrus, conception, cows

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ULCERATIVE MAMARY DERMATITIS AT DAIRY COWS – ETIOLOGY, PREVALENCE, GROSS PATHOLOGY AND STAGING

ULCERATIVNI MAMARNI DERMATITIS MLEČNIH KRAVA – ETIOLOGIJA, PREVALENCA I PATOLOGIJA

Bojan Toholj[a*], Jovan Spasojević[a], Branislav Kureljušić[b], Ivan Galić[a], Nedim Zahirović[a]

[a]University of Novi Sad, Faculty of Agriculture, Departement of Veterinary medicine, Novi Sad, Serbia
[b]Scientific veterinary institute of Serbia, Belgrade, Serbia
*Corresponding author: bojantoholj@gmail.com

B.T. https://orcid.org/0000-0003-4586-8113, J.S. https://orcid.org/0000-0001-5654-5796, B.K. https://orcid.org/0000-0003-4281-1672, I.G. https://orcid.org/0000-0002-6281-0776

ABSTRACT

Ulcerative mammary dermatitis (UMD) also known as udder cleft dermatis first has been observed in England. Clinically it can be describet as a mammary skin disease which causes moist udder sores with foul smelling, very smilar to digital dermatitis (DD) lesins. Typical localization of UMD is a skin between the forequarter and abdominal wall, but it can also be found at the lower part of forequarter skin between papilas. Samples were collected from s Holstein Friesian dairy herd with recurrent cases of DD. The animals were kept in loose house systems with slatted and element floor. The clinical examination was performed during milking parlour using a lighted mirror device for easy acces and view. Tissue samples was taken from different stages of UMD, and fixed in formalin, and stained HE and WS. We also employed a PCR analysis to determine a Treponema presence in tissue samples. The prevalence of UMD was 4,3%, and always at milking cows, and no case at dry cows. We found all stages of this disease. Patohistological analiysis reveal epidermal thickening, paraceratosis, neutrophil infiltration, WS staining reveals a presence of spiral microorganisms in deep skin layers at lesion. In conclusion UMD can be determined as multifactorial disesase but with important role of Treponemas. Patohistology is characteristic and WS staining can reveal presence of spiral bacteria (Treponemas) in deep layers. Milking cows are more proun to develop this disease than dry cows.

Key words: ulcerative mammary dermatitis, pathology, Troponemas.

SAŽETAK

Ulcerozni mamarni dermatitis (UMD), takođe poznat kao dermatitis rascepa vimena, prvi put je primećen u Engleskoj. Klinički se može opisati kao bolest kože dojke koja uzrokuje vlažne rane na vimenu sa neprijatnim mirisom, veoma slične lezima digitalnog dermatitisa (DD). Tipična lokalizacija UMD je koža između prednje četvrtine i trbušnog zida, ali se može naći i na donjem delu kože prednje četvrtine između papila. Uzorci su prikupljeni iz holštajn frizijskog mlečnog stada sa rekurentnim slučajevima DD. Životinje su držane u rastresitim sistemima sa rešetkastim i elementarnim podom. Klinički pregled je obavljen u muzilištu korišćenjem osvetljenog ogledala za lakši pristup i pregled. Uzorci tkiva su uzeti iz različitih stadijuma UMD, fiksirani u formalinu i obojeni HE i VS. Takođe smo koristili PCR analizu da bismo utvrdili prisustvo Treponema u uzorcima tkiva. Prevalencija UMD je bila 4,3%, i to uvek kod muznih krava, a kod suvih krava nije bilo. Pronašli smo sve faze ove bolesti. Patohistološka analiza otkriva zadebljanje epiderme, paraceratoza, infiltraciju neutrofila, VS bojenje otkriva prisustvo spiralnih mikroorganizama u dubokim slojevima kože na leziji. U zaključku, UMD se može odrediti kao multifaktorska bolest, ali sa važnom ulogom treponema. Patohistologija je karakteristična i VS bojenje može otkriti prisustvo spiralnih bakterija (treponema) u dubokim slojevima. Krave muzare su sklonije razvoju ove bolesti od suvih krava.

Ključne reči: ulcerativni mamarni dermatitis, Troponema, patologija.

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ULCERATIVE MAMMARY DERMATITIS

Ulcerative mammary dermatitis (UMD) also known as udder cleft dermatis first has been observed in England (Blowey and Edmnondsen, 1995). Clinically it can be describet as a mammary skin disease which causes moist udder sores with foul smelling, very smilar to digital dermatitis (DD) lesins (Evans et al., 2010). Typical localization of UMD is a skin between the forequarter and abdominal wall, but it can also be found at the lower part of forequarter skin between papilas. The lesions can also impair animal welfare, milk production, and milk quality, and can lead to premature culling (Riekerink, 2014). The etiology is oftenly described as multifactorial such as udder conformation, DIM and parity (Beattie and Taylor, 2000). Also, a link between UCD and digital dermatitis was suggested in some studies (Boyer and Singleton, 1998; Stamm et al., 2009), whereas other studies have shown a weak link (Beattie and Taylor, 2000), no link (Hansen and Nissen, 2010), or a lower risk for UCD (Warnick et al., 2002) in cows with digital dermatitis compared with cows without digital dermatitis. Reported prevalences were 18% in the United States (Warnick et al., 2002), 22% in 1 Scottish farm and 1.4% in an abattoir survey of 331 culled cows (Beattie and Taylor, 2000), and up to 39% within herd and 18.5% average in Sweden (Persson Waller et al., 2014). The aim of this isnvestigation was to determine a gross patology, histopathology, ethiology and staging of Ulcerative mammary dermatitis. A UCM lesions can be with different loacalization, shape. First staging has been desribet by (Reikernik et al., 2014). He described UCM in stages folowing mostly a clinically presentation of these lessions, but not a histopathological and transition between stages criteria. In our investigation we put focus not only on clinicall (macroscopic shape) but also to the a transition proces between stages (figure 1) an histopathological evidence and pain. We also took in mind that a UCM and DD are caused by simmilar microorganisams, and asumed a simmilarity in pathogenesis.

MATERIAL AND METHODS, RESULTS AND CONCLUSSIONS

Samples were collected from s Holstein Friesian dairy herd with recurrent cases of DD. The animals were kept in loose house systems with slatted and element floor. The clinical examination was performed during milking parlour using a lighted mirror device for easy acces and view. Tissue samples was taken from different stages of UMD, and fixed in formalin, and stained HE and WS. We also employed a PCR analysis to determine a Treponema presence in tissue samples (QIAamp DNA mini kit, Qiagen).
The prevalence of UMD was 4,3%, and always at milking cows, and no case at dry cows. We found all stages of this disease. Pstohistological analyisis reveal epidermal thickening, paraceratosis, neutrophil infilitration, WS staining reveals a presence of spiral microorganisms in deep skin layers at lesion. PCR alnalysis indentified a *T.medium/T.vincentii* grupa *T. phagedenis* grupa and *T. denticola/T. Putidum.*
In conclusion UMD can be determined as multifactorial disesase but with important role of Treponemas. Patohistology is characteristic and WS staining can reveal presence of spiral bacteria (Treponemas) in deep layers. Milking cows are more proun to develop this disease than dry cows.

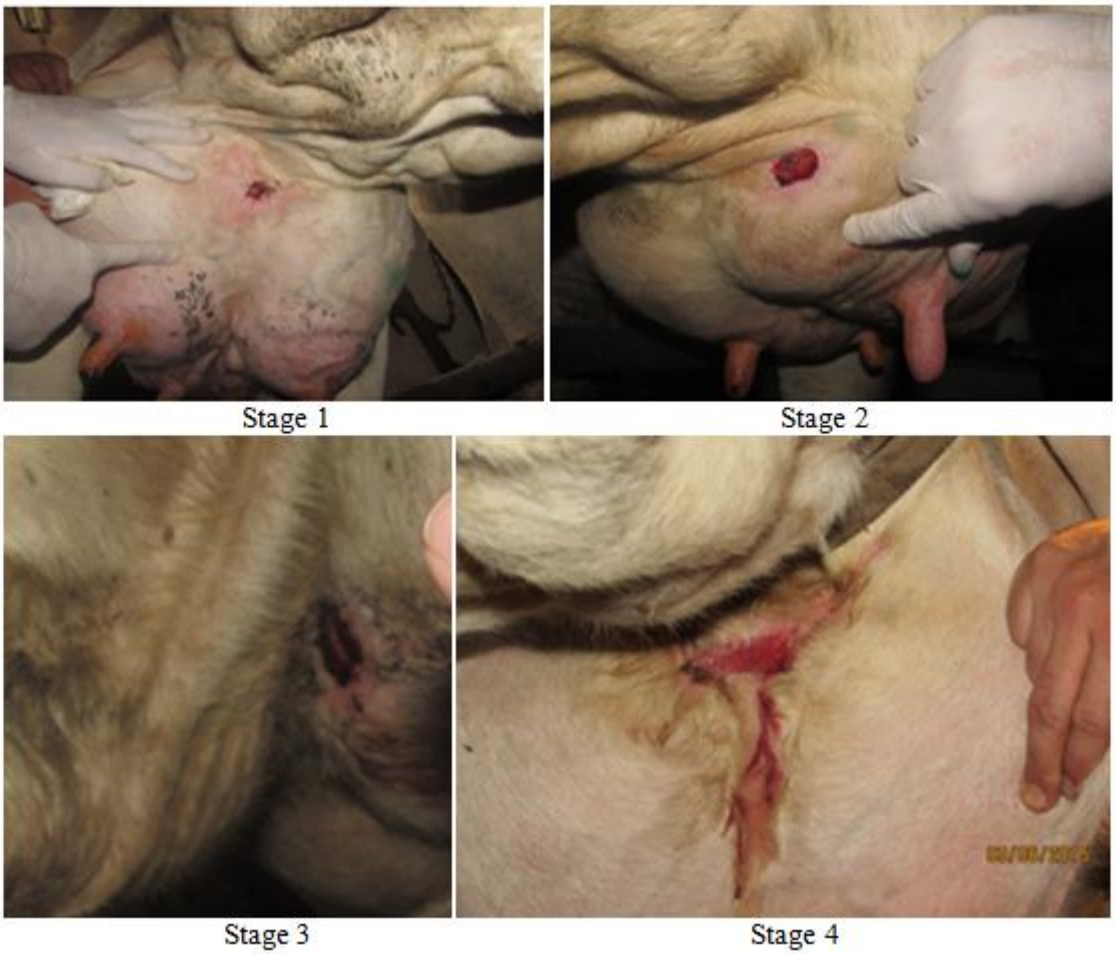

Figure 1. Stages of ulcerative mammary dermatitis

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LITERATURE

1. *Beattie G., Taylor D. An investigation into intertrigo (necrotic dermatitis or 'foul udder') in dairy cows. Cattle Pract. 2000, 8:377–380.*
2. *Boyer P., Singleton G. Digital dermatitis superfoul and severe necrotic dermatitis of the udder in dairy cows. Vet. Rec. 1998, 142:147–148.*
3. *Evans N.J., Timofte D., Carter S.D., Brown J.M., Scholey R., Read D.H., Blowey R.W. Association of treponemes with bovine ulcerative mammary dermatitis. Vet. Rec. 2010, 166:532–533.*
4. *Hansen, M. J., and M. M. Nissen. A Clinical Study of Udder Cleft Dermatitis on a Danish Dairy Farm. Det Biovidenskabelige Fakultet for Fødevarer, Veterinærmedicin og Naturressourcer, Institut for Produktionsdyr og Heste, Copenhagen University, Copenhagen, Denmark, 2010.*
5. *Riekerink O., Amersfort A., Sampimon O., Hooijer G. Prevalence, risk factors, and a field scoring system for udder cleft dermatitis in Dutch dairy herds. J. Dairy Sci. 2013, 97 :5007–5011.*
6. *Stamm L.V., Walker R.L., Read D.H. Genetic diversityof bovine ulcerative mammary dermatitis-associated Treponema. Vet. Microbiol. 2009, 136:192–196.*
7. *Warnick L.D., Nydam D., Maciel A., Guard C.L., Wade S. E. 2002. Udder cleft dermatitis and sarcoptic mange in a dairy herd. J. Am. Vet. Med. Assoc. 221:273–276.*

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DEER FARMING AS PROFITABLE AGRIBUSSINES: THE HIDDEN POTENTIAL IN SERBIA

FARME JELENA KAO PROFITABILAN AGROBIZNIS: SKRIVENI POTENCIJAL U SRBIJI

Miroslav Urošević[a*], Sara Čer[a], Dejan Beukovic[a], Jovan Mirčeta[b], Beata Abramowicz[c], Miroslava Polovinski Horvatović[a]

[a] University of Novi Sad, Faculty of Agriculture, Dep. of Animal Science Novi Sad, Serbia
[b] PE Vojvodinasume, Preradovićeva 2, Petrovaradin-Novi Sad, Serbia
[c] University of Life Sciences in Lublin, Department and Clinic of Animal Internal Diseases, Lublin, Poland
[*]Contact person e-mail: miroslav.urosevic@stocarstvo.edu.rs

M.U. https://orcid.org/0000-0002-8173-5986, S.Č. https://orcid.org/0009-0003-0406-8782, D.B. https://orcid.org/0000-0003-1431-0475, J.M. https://orcid.org/0000-0001-6667-3417, B.A. https://orcid.org/0000-0003-4801-3073, M.P.H. https://orcid.org/0000-0002-0273-197X

ABSTRACT

The farming of deer (*Cervidae* family) has increased globally during the last decades, both in the number of farming operations and the economic output. The increasing interest of consumers in the so-called “free-range products” is reflected in the worldwide development of wild animal farming. Deer are farmed on multiple continents for multiple markets including products such as venison, velvet, urine and antlers. New Zealand is by far the largest exporter of deer meat (venison) and products in the world. Venison equates to 91 percent of the total volume of their deer products exported, however is just over 58 percent of the value. In the United States of America (USA) deer farming as alternative agricultural pursuit is a promising way to preserve the traditional rural way of life while taking advantage of a booming industry. In Europe, production and consumption data for farmed deer are scarce. An exception could be the report of EFSA (European Food Safety Authority) 10 years ago. It stated that approximately 280.000 deer, predominantly Red deer (*Cervus elaphus*) and Fallow deer (*Dama dama*) are farmed in Europe, but less than half of these are slaughtered annually. In Serbia and throughout Europe, venison from deer has increased in popularity and market value owing to its high nutritional value, excellent eating attributes, environmental sustainability, and deep cultural roots. Concomitantly, cattle production has become less profitable and production has decreased over the last two decades leading to protests and economic stagnation in rural Serbia. The low labour regime for deer farming (1 hour work/ per day) means that it can easily complement other livestock and arable enterprises. Dairy cattle farms, with existing buildings, are ideally suited for conversion to deer. In the future, deer farming will increase in Central-, Southeast Europe; smaller farms tend to fallow deer – bigger farms to red deer. Venison is an emerging agro-industry throughout Europe and deer farming has the potential to bolster declining agricultural markets in Serbia. Currently in Serbia all venison is derived from hunted animals, farming does not yet occur. The challenge in Serbia is therefore to harmonize production, regulation and markets with the European Union while making the Serbian venison market equitable, sustainable and profitable. Regulations must be harmonized with European Union, educational programming for deer farmers need to be developed, and venison markets need to be created. As an example, in the Animal welfare law in Serbia it is not present any definition of deer or wild game farm. Consequently, there are no guidelines about the conditions of accommodation, housing, feeding, management and handling. Also, in the Regulation about animal transport there is a lack of rules about the transport of wildlife (or game animals) as well as on the provision of relevant certificate of competence for drivers or handlers. National legislation about meat production in Serbia should consistently define “small quantities”, “local sales” and “direct supply to the final consumer” for the purposes of supply of in-fur carcasses.

Key words: Wildlife farming, Europe, venison, regulation, sustainability.

SAŽETAK

Uzgoj jelena (fam. *Cervidae*) se globalno povećao tokom poslednjih decenija, kako po broju aktivnih farmi, tako i u ekonomskom smislu. Sve veće interesovanje potrošača za takozvane „proizvode iz slobodnog uzgoja u prirodi“ dovelo je do razvoja u gajenju divljih životinja u svetu. Jeleni se uzgajaju na više kontinenata za brojna tržišta, uključujući proizvode kao što su meso, bast, urin i rogovlje. Novi Zeland je ubedljivo najveći izvoznik na svetu mesa i drugih proizvoda od jelena. Tamo, meso divljači predstavlja 91% ukupne količine izvezenih proizvoda od jelena, ali je zato samo 58% u smislu ekonomske vrednostu navedenog izvoza. U Sjedinjenim Američkim Državama (SAD) uzgoj jelena egzistira kao alternativna poljoprivredna delatnost ali je istovremeno put da se očuva tradicionalni ruralni način života uz korišćenje prednosti industrije koja se ubrzano razvija. Nasuprot tome, podaci o proizvodnji i potrošnji uzgajanih jelena u Evropi su oskudni. Izuzetak bi mogao biti izveštaj EFSA (Evropske agencije za bezbednost hrane) od pre 10 godina. U njemu se navodi da se u Evropi uzgaja oko 280.000 jelena, uglavnom Evropskog jelena *(Cervus elaphus)* i jelena lopatara *(Dama dama)*, ali manje od polovine ovog broja godišnje ide na klanje. U Srbiji i širom Evrope meso od jelena postaje sve popularnije uz rast tržišne vrednosti zahvaljujući svojoj visokoj nutritivnoj vrednosti, odličnim gastronomskim svojstvima, ekološkoj održivosti i dugoj tradiciji. Istovremeno, govedarska proizvodnja u Srbiji je sve manje isplativa, a njen obim je opao u poslednje dve decenije što je dovelo do protesta farmera i ekonomske stagnacije u ruralnim područjima. Mala potreba za radnom snagom u gajenju jelena (1 sat rada/dnevno) znači da lako može postati dopunska delatnost na stočarskim i ratarskim farmama. Farme mlečnih krava, sa postojećim zgradama, mogu biti idealno rešenje da se pretvore u farme jelena. U budućnosti se očekuje povećanje uzgoja jelena u Centralnoj i Jugoistočnoj Evropi: farme za gajenje ovaca i koza imaju tendenciju za držanje jelena lopatare, a govedarske farme za Evropskog jelena. Širom Evrope farme divljači su grana agroindustrije u nastajanju i uzgoj jelena ima potencijal da oporavi tržište poljoprivrednih proizvoda u Srbiji. Trenutno se u Srbiji svo meso divljači dobija iz lova, a uzgoj još nije prisutan. Zato predstoje brojni izazovi u Srbiji vezano za usklađivanje proizvodnje, regulative i standarda sa Evropskom unijom, u cilju uređenja srpskog tržišta mesa divljači u održivo i profitabilno. Mora se uskladiti zakonska regulativa sa Evropskom unijom, da se razviju edukativni programi za uzgajivače jelena i uspostavi tržište mesa divljači. Na primer, u Zakonu o dobrobiti životinja u Srbiji ne postoji definicija jelena ili farme divljači. Shodno tome, ne postoje odredbe o uslovima smeštaja, nege, ishrane, menadžmenta i rada sa životinjama. Takođe, u Pravilniku o prevozu životinja nedostaju odrednice o prevozu divljači (divljih životinja) kao i o sticanju odgovarajućeg sertifikata o osposobljenosti za vozače ili radnike koji rade sa divljim životinjama. Nacionalno zakonodavstvo o proizvodnji mesa u Srbiji treba detaljno da definiše „male količine” proizvoda mesa divljači, „lokalni promet” i „direktno snabdevanje krajnjeg potrošača” za potrebe snabdevanja mesom od gajenja divljači.

Key words: Farme divljih životinja, Evropa, meso divljači, propisi, održivost.

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Review
Pregledni rad

HROMOST KOD JUNICA – INTEGRACIJA BIOLOŠKIH, METABOLIČKIH I PROIZVODNIH OSOBINA I AMBIJENTALNIH FAKTORA KAO PREDISPONIRAJUĆIH ZA NASTANAK HROMOSTI

LAMENESS IN HEIFERS - INTEGRATION OF BIOLOGICAL, METABOLIC AND PRODUCTION CHARACTERISTICS AND ENVIRONMENTAL FACTORS AS PREDISPOSING FOR THE OCCURENCE OF LAMENESS

Nedim Zahirović[a*], Bojan Toholj[a], Marko Cincović[a], Ozren Smolec[b], Mimi Ristevski[c]

[a]Departman za veterinarsku medicinu, Poljoprivredni fakultet, Univerzitet u Novom Sadu, Srbija
[b]Veterinarski fakultet, Sveučilište u Zagrebu, Hrvatska
[c]Veterinarski fakultet Bitolj, Univerzitet "Sv.Kliment Ohridski", Severna Makedonija
*Autor za kontakt: nedimzahirovic4@gmail.com

B.T. https://orcid.org/0000-0003-4586-8113, M.C. https://orcid.org/0000-0002-5683-8983, O.S. https://orcid.org/0009-0002-1987-9513, M.R. https://orcid.org/0009-0009-7836-7842

SAŽETAK

Značaj hromosti kod junica je u poslednje vreme sve više prepoznat pa je tako izneta teza da je „hromost kod krava oboljenje koje počinje kod junica". U svom radu navodi da nastanak hromsoti kod junica ih predisponira i kasnije za češće nastajanje oboljenja akropodijuma koja mogu dovesti do hromosti. Rana detekcija hromosti ili još bolje rano otkrivanje predisponirajućih faktora kod junica, moglo bi da ima veliki značaj u kvalitetnijem sagledavanju problematike hromosti kod goveda. Korijum papka koji je oštećen nekim oboljenjem gubi svoje primarne karakteristike a pre svega svojstvo ublažavanja pritiska, zbog toga promene koje nastaju usled oboljenja koja dovode do hromsti, čine da goveda budu podložnija za nastanak hromosti u budućnosti. Opisane su i permanenetne promene na papčanoj kosti koje deluju na nastanak pojačanog pritiska na korijum i češći nastanak hromosti. Praktično, hromost predstavlja faktor rizika za ponovni nastanak hromosti. Ovo je naročito važno kod junica jer ukoliko hromost nastane u ranoj fazi života, onda će i epizode hromosti biti učestalije u ostalom periodu. Cilj istraživanja je ispitati uticaj kliničkih, morfoloških, biohemijskih i hematoloških parametara kod junica u periodu rasta i razvoja na mogućnost predikcije nastanka hromosti nakon teljenja.

Ključne reči: junice, predisponirajući faktori, hromost.

ABSTRACT

The importance of lameness in heifers has been recognized more and more recently, so the thesis that "lameness in cows is a disease that starts in heifers" has been presented. In his work, he states that the occurrence of chromosotis in heifers predisposes them later to more frequent occurrence of acropodium diseases that can lead to lameness. Early detection of lameness, or even better, early detection of predisposing factors in heifers, could be of great importance in better understanding the problem of lameness in cattle. The corium of the hoof that is damaged by some disease loses its primary characteristics, and above all the property of relieving pressure, therefore the changes that occur due to diseases that lead to lameness, make cattle more susceptible to lameness in the future. Permanent changes on the palpebral bone have also been described, which lead to increased pressure on the corium and more frequent lameness. Practically, lameness is a risk factor for the recurrence of lameness. This is especially important in heifers because if lameness occurs in the early phase of life, then episodes of lameness will be more frequent in the rest of the period. The goal of the research is to examine the influence of clinical, morphological, biochemical and hematological parameters in heifers during the period of growth and development on the possibility of predicting the occurrence of lameness after calving.
Keywords: heifers, predisposing factors, lameness.

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TEHNOLOGIJA GAJENJA JUNICA I SKLONOST KA HROMOSTI

Biologija i uzgoj junica predstavljaju veoma značajnu kariku u procesu dobijanja jedinki koje mogu biti dovoljno produktivne i dovoljno zdrave, kako bi se onemogućile direktne ekonomske štete od preranog isključivanja životinja sa farmi ili različitih bolesti. Uzgoj junica je opisan u mnogim klasičnim knjigama o stočarstvu, tehnologiji i ishrani goveda (1-5). Junice se drže u grupama koje bi trebale da imaju od 15 do 20 životinja, što je dobro sa etološkog aspekta kako bi se navikle na hijerarhiju i sa aspekta epidemiološke stabilnosti i nastanka, odnosno širenja zaraznih bolesti u objektu. Grupe se formiraju prema dobu životinja i telesnim osobinama, a različite grupe prati i različita receptra ishrane. Pri grupnom uzgoju preporučuje se formiranje sledećih starosnih grupa sa određenim tehnološkim osobinama ishrane: a) grupa junica od 5-6 meseci života, koje će se hraniti prelaznim režimom ishrane i u zimskom i u letnjem periodu; b) grupa junica od 7-12 meseci života. To su grla koja se privikavaju na ishranu kabastim krmivima; koncentrat se daje leti i zimi prema potrebi i kvalitetu kabastih krmiva; c) grupa junica od 13-14 meseci, koje se hrane kabastim krmivima, ako ova kvalitetom odgovaraju postavljenim normama, treba davati manje količine koncentrata; d) grupa junica od 15 do17 meseci, koje su određene za pripust, a hrane se isključivo kabastim krmivima, ako su kvalitetna. Treba pojačati koncentrat prema normativima; e) grupa junica od osemenjavanja pa sve do šestog meseca gravidnosti; f) visoko gravidne junice, gravidne u 6. mesecu i dalje, koje se intenzivnije hrane i zbog toga dobijaju veće doze koncentrata a spremaju se za telenje.

Kada se radi o zoohigijenskom aspektu ishrane junica veom je značajna dužina jasala koju treba obezbediti po jedinki, pa tako za kategorije u starosnoj dobi od 5 do 12 meseci trebalo bi po junici obezbediti 50 cm dužine jasala, za kategoriju od 13 do 18 meseci oko 60 cm i za grupu starosti od 19 do 24 meseca 70 cm. Pored ovoga potrebno je obezbediti određene uslove sa aspekta structure obroka i kvaliteta hraniva. Struktura obroka mora biti takva da omogući radi razvoj predželudaca i creva, pa bi obrok trebao da sadrži oko 2 kg suve materije na 100 kg telesne težine junice, sa 200-300 grama vlakana na kilogram suve mase, da bi se omogućio rad buraga. Dobar izbor bi mogao biti ishrana travnom silažom kao osnovom, a ova silaža bi morala imati u 1 kg suve materije najviše do 200 g sirovih vlakana i najmanje 125 g sirovog proteina. Koncentrat koji se daje junicama bi trebao da ima sledeće karakteristike hranljive vrednosti: svarljivi protein 13,5-14% zimi odnosno 9,2-9,5% leti, a krmnih jedinica 1,1-1,15 zimi odnosno 1,0-1,05 jedinica leti. Kvalitet kabaste hrane treba konstantno proveravati i ispitivati i potrebno je da se koncentrovanom hranivom izbalansiraju određeni nedostaci kabaste hrane, posebno sa aspekta kvalitativnog mineralnog sastava, ali i drugih kvalitativnih ingredijenata. Junice bi trebale da dobijaju koncentrat konstatno od šestog do desetog ili dvanaestog meseca života, a potom se koncentrat daje zavisno od kvaliteta ostalih krmiva, a može se gotovo izostaviti iz obroka sve do sedmog meseca graviditeta. U poslednjem trimestu graviditeta treba polagano opet uvoditi koncentrat u obrok do količine koja je propisana kao dnevna norma za prosečnu životinju u grupi, ako se radi o grupnom hranjenju. U prvoj polovini graviditeta praktično nema povećanja hranljivih potreba, ali se potrebe značajno povećavaju posle ovog perioda zbog intenzivnog porasta ploda, ali i činjenice da će posle teljenja junice ući u metabolički izuzetno zahtevan početak laktacije, pa je potrebno izbeći suviše veliko opadanje energetskog bilansa i mnoge pridružene bolesti kao što su ketoza, mastitis, metritis ili razne bolesti papaka. Ukoliko se ne vrši adekvatno povećanje obroka junice će ostati nepriviknute na količine hrane koju bi trebale normalno da konzumiraju posle teljenja, što se može odraziti na smanjenu proizvodnju mleka. Tako junice mogu da konzumiraju gotovo 4 kg koncentrata dnevno, a količina stočne hrane se smanjuje oko nedelju dana pred očekivano teljenje, a koncentrat se nekoliko dana pred teljenje može i izbaciti. Potrebno je smanjiti neposredno pred teljenje i davanje sena, da bi se smanjilo punjenje digestivnih organa, a sva snaga trbušne prese se usmerila na istiskivanje ploda i partus. Po teljenju junicama treba dati topao napoj od mekinja, a potom odmah krenuti sa laganim povećavanjem količine sena u narednih nekoliko dana. Potrebno je u prvim nedeljama laktacije davati apetibilnu hranu koje životinje rado jedu, a cilj je da se postigne maksimalni unos kabaste, a za njom i koncentrovane hrane u prcih tri do četiri nedelje po teljenju.

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BIOLOŠKE I METABOLIČKE OSOBINE JUNICA I SKLONOST KA HROMOSTI

Sledeći bitan aspekt su biološke osobine junica koje utiču na reprodukciju. U trenutku prvog osemenjavanja junice bi trebale da dostignu oko 60% normalne mase odrasle krave i trebale bi da imaju masu od oko 400 kg. Polovina očekivane telesne težine (a to je 300 kg) najveći broj jedinki dostigne sa oko godinu dana starosti. To znači da bi od rođenja junice trebale prosečno da napreduju oko 750 g dnevno. U tom smislu mora se voditi računa da junice budu normalno uhranjenje, ali da ne prelaze u tovnu kondiciju, što negativno utiče na reprodukctivnu efikasnost. Pored telesne mase smatra se da bi u momentu osemenjavanja u obzir trebalo uzeti i visinu životinje, pa bi visina krsta prilikom prvog osemenjavanja trebala da bude 1,3 m, a što ukazuje na postignutu telesnu masu od 400 kg ±10%. Prerano osemenjavanje dovodi do problema koa što su niži stepen steonosti junica koje su tek postigle polnu zrelost, veći problemi pri telenju, kao i manja mlečnost u prvoj laktaciji. Takođe velika je greška da se posle osemenjavanja junice koje nisu postigle adekvatne telesne mere dodatno hrane, jer će na taj način sasvim sigurno ući u gojaznost, a potom i biti sklonije ka lipolizi i ketogenezi kako se približava partus i laktacija. Smatra se da će se postići viša proizvodnja mleka za oko 60 litara u laktaciji, ako se prvo osemenjavanje prolongira za mesec dana, ali suviše kasno osemenjavanje iako će dovosti do veće proizcodnje mleka u prvoj laktaciji remeti dalje proizvodne cikluse, pa se ova prednost brzo gubi.

Kod bioloških osobina junica potrebno je utvrditi metaboličke pokazatelje junica u ranom periodu i metaboličke pokazatelje junica u kasnijem gravidnom periodu, a posebno u funkciji telesne kondicije. U jednom našem istraživanju ispitivali smo metaboličke adaptacije i porast u ranom periodu junica kada su one stare 6- 12 meseci (6). Za ocenu telesnog porasta kod junica u praksi se koriste različite telesne mere, od merenja telesne težine, do linearnih mera kao što su obim grudi, visina grebena, dužina tela ili telesna kondicija. Međutim, istraživanja u poslednjih 15-ak godina pokazuju da je neophodno da se pored telesnih mera uključe i različiti metabolički pokazatelji. Cilj ovog rada bio je da se ispita uticaj starosti junica na karakteristike linearno izmerenih telesnih mera i parametara iz metaboličkog profila. U ogled je uključeno 105 junica starosti 6-12 meseci, pre puberteta i prvog osemenjavanja. Rezultati istraživanja pokazuju da je vrednost obima grudi iznosila 150,96±13,92 cm, dok je dužina tela iznosila 120,71±10,93 cm. Vrednost navedenih parametara raste sa starošću životinja. Nije nađena signifikantna linearna povezanost između starosti i telesnih mera junica. Postoji pozitivna korelacija između obima grudi i dužine tela junica, sa koeficijentom determinacije R^2= 0,498 (p<0,01). Starost pokazauje značajan uticaj na sledeće metaboličke parametre: albumin, ukupni bilirubin, glukoza, P, holesterol, trigliceridi, BHB i NEFA. Kod junica tokom vremena dolazi do opadanja vrednosti albumina, glukoze, triglicerida, NEFA i BHB, a dolazi do porasta vrednosti holesterola i bilirubina. Ove razlike su najupečatljivije kada se uporede junice starosti 6 i 12 meseci. Nije pokazan signifikantan uticaj telesnih mera na metaboličke parametre kod junica starosti 6-12 meseci.

Kod junica se dešavaju metaboličke promene slične homeorezi kod krava višeg pariteta, a odnosi se na lipolizu, ketogenezu, insulinsku rezistenciju i promenu u energetskom metabolizmu. U jednom našem istraživanju (7) utvrđeno je da li postoji razlika u bazalnim koncentracijama glukoze, insulina, NEFA i indeksa insulinske rezistencije RQUICKI, glukoza:insulin i NEFA:insulin odnos kod junica u funkciji telesne građe i gravidnosti. U ogled je uključeno 40 junica Holštajn-frizijske rase koje su bile klasifikovane u 4 grupe: 1) negravidne junice normalne telesne kondicije, 2) negravidne junice visoke telesne kondicije (gojazne), 3) gravidne junice nornalne telesne kondicije i 4) gravidne junice visoke telesne kondicije (gojazne). Negravidne junice su bile u postpubertalnom periodu (preko 150 kg telesne mase), a gravidne su bile u drugom trimestru gravidnosti. Gojazne junice u poređenju sa junicama normalne telesne kondicije imaju insulinsku rezistenciju koja se odlikuje povećanom koncentracijom glukoze pre gravidnosti i sniženom tokom gravidnosti, povišenom koncentracijom insulina i NEFA, nižom vrednosti RQUICKI indeksa i nižim odnosom glukoza:insulin. Gravidnost kod junica dovodi do opadanja koncentracije glukoze i insulinske rezistencije masnog tkiva kada raste koncentracija NEFA, a odnos NEFA:insulin je viši. Kada se radi o korišćenim indeksima insulinske rezistencije rezultati pokazuju da je: RQUICKI indeks niži kod gojaznih junica, a da graviditet nema uticaja; odnos NEFA:insulin je viši samo kod gravidnih jedinki, a telesna kondicija nema uticaja, dok se odnos

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glukoza:insulin menja kako pod uticajem telesne kondicije tako i pod uticajem gravidnosti pa je ovaj nodnos najniži kod gravidnih i gojaznih junica. Gojaznost i graviditet kod junica utiču na razvoj insulinske rezistencije.

Gojaznost uz povećan telesni prirast od preko 750g dnevno do 15. meseca starosti predstavlja značajan faktor rizika koji može uticati i na zdravlje papaka. Tako preterana težina gojaznih junica povećava pritisak na sve anatomske strukture akropodijuma koji još uvek nije ni formiran u potpunosti. Kombinacija povećane težine sa posledičnim povećanim pritiskom na papke uz neadekvatnu podlogu objekta direktno predisponira junice ka laminitisu i ostalim poveznim bolestima. Pored ovoga u obzir treba uzeti i činjenicu da starost ima značajan uticaj na strukturu papaka, a pre svega masnih jastučića. Pokazano je da su kod junica jastučići izgrađeni od rastresirog vezivnog tkiva, a količina masnog tkiva se povećava sa storošću. Jasno je da veće prisustvo rastresitog veziva u poređenju sa čvršćom konzistencijom masnog tkiva predisponira junice ka nastanku hromosti. Masno tkivo se u svom masnokiselinskom sastavu menja sa starošću (8). Kod junica ima više zasićenih masnih kiselina koje čine ove jastučiće čvršćim, te bolje drže odnosno amortizuju papčanu kost. Sa druge strane, kod krava ima više nezasićenih masnih kiselina. Ta izmena masnokiselinskog sastava dodatno u fokus stavlja metabolizam i zdravlje junica, te ranu detekciju hromosti junica kao bitan faktor rizika za dalji nastanak hromosti tokom prve i narednih laktacija. Promene u metabolizmu lipida u peripartalnom periodu mogu biti veoma značajan faktor zdravlja papaka, što opotvrđuju određena podudaranja iz epidemioloških studija, pa tako se povećanje patologije papaka dešava najviše u prvih sto dana posle teljenja kada jastučići sadrže manje masnog tkiva koje je zamenjeno kolagenim (9).

Metabolizam ugljenih hidrata i dostupnost šećera je veoma značajan u očuvanja zdravlja papaka. U eksperimentalnim modelima preopterećenja ugljenim hidratima moguće je izazvati hromost kod junica posebno zbog izazivanja bola čak i kod negravidnih junica. Negravidne junice pate od bola i razvijaju senzacije identične kao i krave što se pokazalo na eksperimentalnom modelu izazivanja hromosti pomoću preopterećenja oligofruktozom kod mlečnih junica (10). Klinički parametri i uzorci krvi dobijani su 48 i 24 h i 6, 12, 24, 36 i 48 h nakon indukcije hromosti. Klinički parametri uključivali su broj otkucaja srca, brzinu disanja, učestalost u rumenu i rezultat hromosti. Biomarkeri u plazmi su uključivali kortizol, haptoglobin, norepinefrin, beta-endorfin i supstancu P. Uočene su razlike u svim parametrima između kontrolne i tretirane junice. Koncentracija biomarkera u plazmi je značajno porasla kod tretiranih životinja počevši od 6 h nakon indukcije hromosti, dostižući maksimalne nivoe za 24 h za kortizol, 48 h za haptoglobin, 6 h za norepinefrin, 12 h za supstancu P i 24 h za beta-endorfin. Ovi rezultati nesumljivo povezuju značaj ugljenih hidrata u procesu nastanka hromosti i razvoja bolnog procesa kod junica.

U našim istraživanjima (21) utvrđena je korelacija između krvnih parametara sa LS je takođe utvrđena kod junica. ALB je pokazao negativnu korelaciju sa LSM4, LSM5, LSMAv i sa pojavom junica koje imaju probleme sa hromošću kao što su visoko skor šepavosti na nivou 4 ili 5 i kod kojih su dva uzastopna merenja viša od 2. GLOB pokazuje pozitivnu korelaciju sa LSM3, LSM4, LSM5, LSMAv i sa pojavom junica koje imaju probleme sa hromošću kao što su visoko skor šepavosti na nivou 4 ili 5 i kod kojih su dva uzastopna merenja viša od 2. TBIL i AST pokazuju statistički značajnu pozitivnu korelaciju sa gotovo svim LS i sa pojavom junica koje su imale problema sa hromošću. Koeficijent korelacije je bio u rasponu od 0,2 do 0,45, a utvrđena je statistička značajnost na nivou $p< 0,01$ za većinu veza. GLU pokazuje pozitivnu korelaciju sa svim LS (osim sa LSM1) i svim kategorijama problematičnih krava postignut je nivo korelacije oko 0,25 uz statističku značajnost $p< 0,01$ za većinu veza. Ca pokazuje pozitivnu vezu sa LSAv kao i sa pojedinim kategorijama problematičnih junica (one koje imaju prosečnu LSAv preko 2, koje su barem jednom imale LS=3 i koje su u dva uzastopna merenja imali LS veći od 2), a ove korelacije su bile statistički značajne od $p< 0,05$ do $p< 0,01$. CHOL negativno korelira sa LSM5, LSMAv i sa junicama koje su barem jednom imale LS 4 ili 5 i koje su u dva uzastopna merenja imale LS veći od 2), a ove korelacije su bile statistički značajne od $p< 0,05$ do $p< 0,01$. Krvni parametri kao što su NEFA, WBC i Gr (koji se ogleda u broju neutrofila) pokazuju pozitivnu korelaciju sa svim ispitanim parametrima pepavosti, tako da je postignut veoma visok statistički prag značajnosti na nivou $p< 0,01$. Hematološki parametri crvene krvne loze nisu pokazali značajnu vezu sa šepavošću kod junica. PLT (trombociti) pokazuju pozitivnu korelaciju sa LSM1. Pogledati Grafikon 1.

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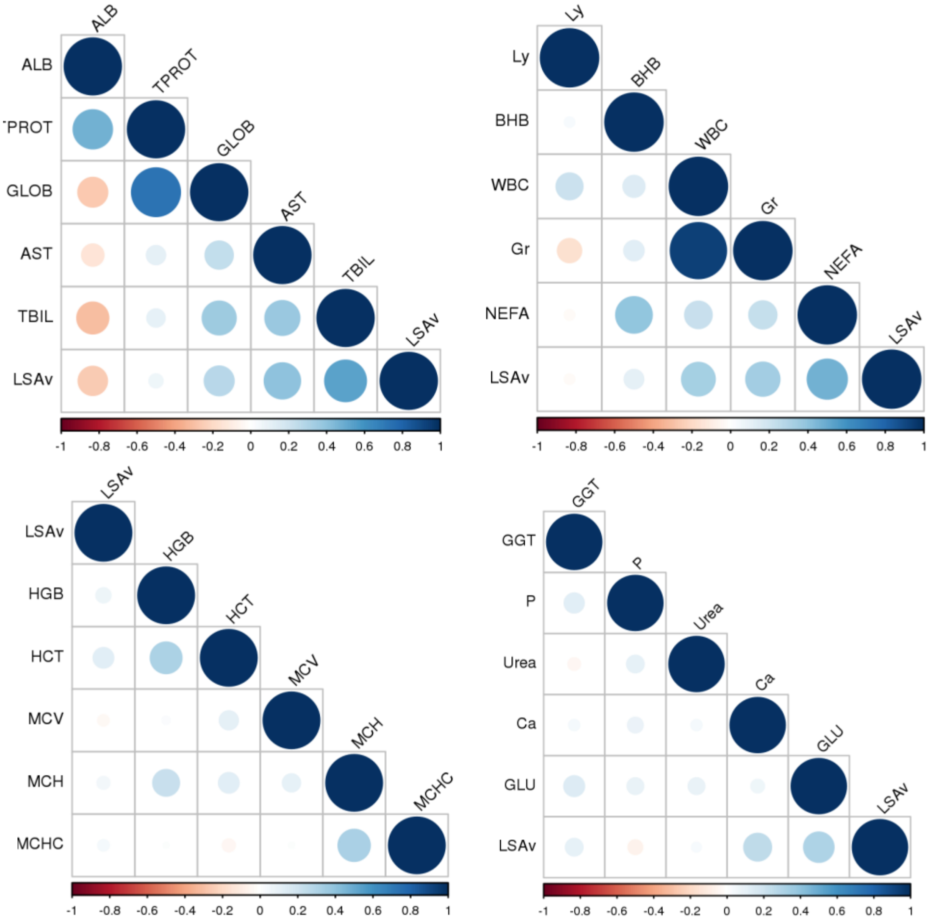

Grafikon 1. Međusobna korelacija ispitivanih krvnih parametara i njigova veza sa prosečnom LS (LSAv).

HROMOST KOD JUNICA I NJEN UTICAJ NA NASTANAK HROMOSTI U STARIJIM ŽIVOTNIM DOBIMA TOKOM LAKTACIJA

Hromost kod junica ima višestruki značaj za njen celokupni životni vek u fazi mlečne krave, kao i zdravlje papaka, posebno u prvih nekoliko laktacija, kada su krave ujedno i najpoduktivnije. Prva pojava hromosti povećava budući rizik od hromosti (11), pa to znači da pojava hromosti kod junica može imati dalekosežne uticaje na celokupan produktivni život u kasnijim paritetima. Ovo je posebno značajno kada se ima u vidu visoka prevalencija lezija kod junica (12,13): prevalenca umerenog do teškog krvarenja tabana je 55%, lezija bele linije 72%, digitalnog dermatitisa dostigla je vrhunac na 39 % u prvih sto dana laktacije, dok 95% svih jedinki u periodu od 50 do 80 dana posle teljenja imaju neku patologiju na najmanje jednom papku.

Boravak junica na otvorenom sa slamenom prostirkum imale su manje krvarenja na tabanskoj regiji u odnosu na junice koje su provele zadnjih mesec dana pred teljenje u boksu (14). Dakle praksa smeštaja junica per teljenja značajno utiče na razvoj inicijalnih lezija papka, koje će doprineti bolesti papaka i hromosti, te dugotrajni efekat na papke.

Kada se radi o dugotrajnom delovanju lezija papaka na buduće zdravlje papaka pokazano je da su teže lezije bele linije i lezije tabana bile povezane sa značajno povećanim rizikom od budućeg hromosti za 1,6 odnosno 2,6 puta u svim budućim laktacijama u stadu (15). Ovaj nalaz se može objasniti nezavisnim rezultatima dobijenih pomoću kompjuterske tomografije, koji su pokazali da lezije u rogovima papaka tokom života zapravo poveze sa trajnim narušavanjem koštane osnove, odnosno koštane arhitekture pedalne kosti (16). Utvrđen je i jedan kompenzatorni mehanizam koji je zanimljiv

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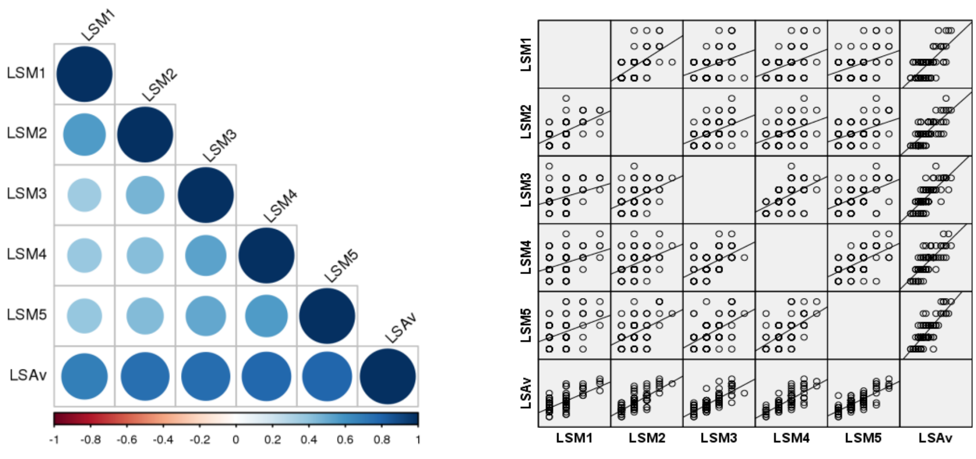
kod junica pa tako blage lezije tabana i bele linije koje su se javile u periodu od 2 do 4 meseca nakon teljenja ili 0 do 2 meseca pre teljenja, bile su povezane sa smanjenim rizikom od hromosti (15). Autori su diskutovali ovakav rezultat kroz svoju sugestiju da određeni stepen blage povrede u vreme prvog teljenja može biti od koristi za dugoročno zdravlje papaka; ako dođe do adaptivnih promena kao odgovora na povredu tokom vremena kada je papak u stanju da se oporavi čime će da postane biomehanički otporniji, a samim tim životinja može biti manje sklona hromosti na duži rok. U prilog ovome govori i činjenica da su najmanju prevalenciju hromosti i sa njom povezanih bolesti papaka imale upravo one krave koje su u periodu pre prvog teljenja, dakle kao gravidne junice boravile na tvrdoj betonskoj podlozi, a u periodu posle teljenja su smeštene na meke površine u vidu posebnih gumenih lamela (17). Boravak na tvrdoj podlozi tokom perioda uzgoja rezultirao traumatskim krvarenjem tabanske regije, ali kako su junice u to vreme bile u stanju da se izbore, to je na kraju bilo korisno za zdravlje papaka. Pored navedenog, pokazano je da izlaganje umerenom pritisku i fiziškoj aktivnosti može dovesti do značajnog poboljšanja potporne strukture akropodijuma, što može imati dugoročne pozitivne efekte (18,19).

Neophodno što ranije prepoznati faktore rizika i bolesti papaka, čak i pre prvog teljenja zbog rizika za nastanak hromosti i sledećih bolesti papaka kasnije tokom laktacije i u narednim laktacijama. Međutim, hromost kod junica i sama po sebi ima i direktne negativne posledice kao i kod krava u višim laktacijama. Kao promer negativnog efekta navodimo inflamatorni odgovor i izmene u metabolitima proteina kod junica sa hromošću (20). Autori su našli da kod junica sa hromošću postoji značajno viša koncentracija haptoglobina, serum amiloida A i fibrinogena u poređenju sa zdravom kontrolom. Kod junica sa bolestima papaka utvrđena je i značajno veća koncentracija ukupnih proteina, a koncentracija kreatinina i uree je bila značajno niža kod krava sa bolestima papaka, a nije postojalo odstupanje u vrednosti albumina i ukupnih globulina. Navedeno istraživanje pokazuje da kod junica postoji direktan uticaj bolesti papaka na krvne parametre, jer su nađena određena odstupanja u rutinskim laboratorijskom parametrima ukoliko postoje bolesti papaka.

Naša ispitivanja (21) su pokazalada postoji statistički značajna pozitivna korelacija između svih vrednosti LS od prvog do petog meseca kao i visoka korelacija pojedinačnih LS sa prosečnim LSAv. Korelacije su bile srednjeg do jačeg intenziteta, a njihova vrednost kretala se od 0,346-0,793 uz statističku značajnost p<0,001. Rezultati su prikazani na Grafikonu. Pored navedenog utvrđena je pozitivna korelacija između LS1-5 i LSAv sa pojavom krava koje će imati problema sa šepavošću. Ta korelacija je pozitivna i kretala se u rasponu od 0,326-0,775 (p<0,001). Rezultati su prikazani u Grafikonu 2. Navedeni rezultati ukazuju da što je veći skor šepavosti u prvom i ranijim mesecima lektacije, to će skor šepavosti biti viši i kasnije do petog meseca laktacije, biće veći prosečan lokomotorni skor kao i učešće junica koje će imati problema sa hromošću.



Grafikon 2. Korelogrami i regresione linije za ispitivanje veze između između lokomotornog skora junica od prvog do petog meseca ispitivanja (LSM1-5) kao i prosečnog lokomotornog skora (LSAv)

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LITERATURA

1. *Mitić N., Ferčej J., Zeremski D., Lazarević L.J. Govedarstvo. Zavod za udžbenike i nastavna sredstva, Beograd, 1987.*
2. *Čobić T., Antov G. Govedarstvo: proizvodnja mleka. Poljoprivredni fakultet, Novi Sad, 1996.*
3. *Romčević L., Trifunović G., Lazarević L. Govedarstvo Srbije. Univerzitet u Beogradu-Poljoprivredni fakultet, 2007.*
4. *Petrović M.D., Petrović M.M., Kurćubić V.S. Govedarstvo-tehnologija proizvodnje, Čačak, 2006.*
5. *Jovanović R., Dujić D., Glamočić D. Ishrana domaćih životinja. Poljoprivredni fakultet Novi Sad, 2001.*
6. *Zahirović N., Galić I., Lakić I., Toholj B., Stančić I., Petrović M., Cincović, M.R. Metabolički status junica uzrasta šest do dvanaest meseci, Letopis naučnih radova 2018, 42, 2, 78-83.*
7. *Cincović M., Belić B., Lakić I., Galić I., Zahirović N., Toholj B., Stančić I., Todorović S. Uticaj telesne kondicije i graviditeta na insulinsku rezistenciju kod junica. Letopis naučnih radova 2019, 43, 2, 157-163.*
8. *Raber M., Scheeder M.R.L. The influence of load and age of the fat content and the faty acid profile of the bovine digital cushion.In: Shearer,J.: Proceedings of the 12th International Symposium of lameness in Ruminants 2002;194.*
9. *Smilie R.H, Hoblet K.H. Subclinical laminitis in diary cows:use of severity of hoof lesions to rank and evalute herds.Veterinary Record 1999, 17-21.*
10. *Bustamante H.A., Rodriguez A.R., Herzberg D.E., Werner M.P. Stress and pain response after oligofructose induced-lameness in dairy heifers. Journal of Veterinary Science 2015, 16(4), 405-411.*
11. *Hirst W.M., Murray R.D., Ward W.R., French N.P. A mixed-effects time-to-event analysis of the relationship between first-lactation lameness and subsequent lameness in dairy cows in the UK. Preventive veterinary medicine 2002, 54(3), 191-201.*
12. *Maxwell O.J.R., Hudson C.D., Huxley J.N. Effect of early lactation foot trimming in lame and non-lame dairy heifers: a randomised controlled trial. Veterinary Record 2015, 177(4), 100.*
13. *Capion N., Thamsborg S.M., Enevoldsen C. Prevalence and severity of foot lesions in Danish Holstein heifers through first lactation. The Veterinary Journal 2009, 182(1), 50-58.*
14. *Webster A.J.F. Effects of housing practices on the development of foot lesions in dairy heifers in early lactation. Veterinary Record 2002, 151(1), 9-12.*
15. *Randall L.V., Green M.J., Chagunda M.G., Mason C., Green L.E., Huxley J.N. Lameness in dairy heifers; impacts of hoof lesions present around first calving on future lameness, milk yield and culling risk. Prev Vet Med. 2016, 133:52-63.*
16. *Newsome R., Green M. J., Bell N.J., Chagunda M.G.G., Mason C.S., et al. Linking bone development on the caudal aspect of the distal phalanx with lameness during life. Journal of Dairy Science 2016, 99(6), 4512-4525.*
17. *Bergsten C., Telezhenko E., Ventorp M. Influence of soft or hard floors before and after first calving on dairy heifer locomotion, claw and leg health. Animals 2015, 5(3), 662-686.*
18. *Knott L., Tarlton J.F., Craft H., Webster A.J.F. Effects of housing, parturition and diet change on the biochemistry and biomechanics of the support structures of the hoof of dairy heifers. The Veterinary Journal 2007, 174(2), 277-287.*
19. *Gard J.A., Taylor D.R., Wilhite D.R., Rodning S.P., Schnuelle M.L., Sanders R.K., et al. Effect of exercise and environmental terrain on development of the digital cushion and bony structures of the bovine foot. American Journal of Veterinary Research 2015, 76(3), 246-252.*
20. *Tóthová C., Nagy O., Seidel H., Paulíková I., Kováč G. The influence of hoof diseases on the concentrations of some acute phase proteins and other variables of the protein profile in heifers. Acta veterinaria 2011, 61(2-3), 141-150*
21. *Zahirović N., Toholj B., Smolec O., Cincović M. The Influence of Body and Metabolic Parameters in the Period Before Sexual Maturation in Heifers on the Lameness Score During the First Lactation. Kafkas Univ Vet Fak Derg, 2024, DOI: 10.9775/kvfd.2024.31462.*

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RADIONICA: PROIZVODNJA GOTOVIH MIKROBIOLOŠKIH HRANLJIVIH PODLOGA, NUTRIENT SUPPLEMENT MEDIUMA I VIRUSNIH TRANSPORTNIH MEDIUM SISTEMA – OD FARME OVNOVA DO FABRIČKOG POGONA, KONTROLE I SISTEMA KVALITETA U "PROMEDIA" OKRUŽENJU

Milan Jovanović[a*], Marko Cincović[b]

[a]ProMedia, Kikinda
[b]Departman za veterinarsku medicinu, Poljoprivredni fakultet, Univerzitet u Novom Sadu
*Autor za kontakt: milan.jovanovic@promedia.rs

M.C. https://orcid.org/0000-0002-5683-8983

ORGANIZACIJA RADIONICE

Sat 1 – Obilazak proizvodnih pogona, analiza kritičnih kontrolnih tačaka i hematološka fiziologija i patofiziologija kao osnov za dobijanja kvalitetne životinjske krvi kao primarne sirovine
Sat 2 – Proces rada u laboratoriji za internu kontrolu kvaliteta
Sat 3 – Sistem kvaliteta i prilagođavanje modernim poslovnim zahtevima

PROIZVODNJA GOTOVIH KRVNIH MIKROBIOLOŠKIH MEDIUMA I KRVNIH SUPLEMENATA

Serijska proizvodnja pune krvi, kao suplementa u mikrobiologiji je izuzetan izazov ako se uzme u obzir da je u pitanju veoma specifičan proizvod životinjskog porekla. Ovaj proizvod je u in-vitro uslovima krajnje osetljiv na promene uslova čuvanja i podložan kontaminaciji, a njegove osobine u pogledu sastava, broja i stanja ćelija, treba održavati u usko standardizovanim granicama. Kao takva, na žalost, puna krv i dalje, u trećoj deceniji 21. veka, nema adekvatnu zamenu. Informacije i literatura o samoj tehnologiji prikupljanja i obrade krvnog produkta su izuzetno skromne i ograničene. Svedene su na strogo čuvane patentne tajne malobrojnih proizvođača u svetu, pa samim tim, činjenica da smo u Srbiji prvi i jedini serijski proizvođači, čini ovaj izazov još većim jer je u pitanju pionirski poduhvat.

ŠTa se sve nalazi iza proizvodnje jedne krvne ploče ili boce krvnog suplementa? Pre svega, mnogo rada i znanja, godine razvoja, zahtevni standardi proizvodnje i prometa medicinskih sredstava, moderna oprema i infrastruktura, rigorozna kontrola, sledljivost od prve do poslednje faze ciklusa proizvodnje i na kraju i na početku, posvećeni stručni kadar. Sledi opis osnovnih proizvodnih faza:

1) PRIKUPLJANJE KRVI OD DONORA (OVACA I KONJA) NA FARMI

Primarna krv (ovčija i konjska) se prikuplja od životinja – donora na našoj specijalizovanoj farmi ovnova ili ovlaštenim farmama sa kojima imamo ugovor.

Životinje na našoj farmi su pod posebnim dijetetskim režimom i konstantnim veterinarskim nadzorom.

Pored obezbeđenih svih neophodnih dozvola nadležnih entiteta, potrudili smo se da obezbedimo najbolje moguće uslove za boravak životinja, a njihovo čuvanje, nega, manipulacija, kao i prikupljanje krvi se odvijaju u skladu sa obavezama definisanim zakonom o dobrobiti životinja.

2) KRVNI DERIVAT

Na farmi su obezbeđeni svi neophodni tehnički uslovi da se krv prikuplja bezbedno i u količinama koje ne ugrožavaju zdravlje jedinke. Obučeni operateri, uz nadzor veterinara, prikupljaju krv, kojoj je posebnom aseptičnom procesu izdvojen fibrin i sačuvana sterilnost.

Primarna krv – krvni derivat, se u kesama i bocama, odlaže u frižidere i sisteme koji održavaju temperature od 2-8°C, što je presudno, naročito kada su spoljne temperature ispod nule ili preko 30°C.

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3)TRANSPORT KRVNOG DERIVATA NA POSEBNOM TEMPERATURNOM REŽIMU

U narednoj fazi se primarni uzorci, propisno obeleženi i sa propratnom dokumentacijom, transportuju do proizvodnog pogona i kontrolne laboratorije u sistemu hladnog lanca, koji se neće prekidati do kraja životnog ciklusa proizvoda.

4) PRIJEM KRVNOG DERIVATA I SKLADIŠTENJE

U Proready proizvodnom pogonu, vrši se prijem krvnog derivata, a zatim i dalja manipulacija i uzorkovanje. Krvni derivat se skladišti u prostoru gde se održava temperatura od 2-8°C.

5) KONTROLA KVALITETA SIROVINE

U našoj internoj specijalizovanoj laboratoriji, obavlja se uzorkovanje svake serije za kontrolu kvaliteta i sterilnosti.

6) PROIZVODNJA KRVNIH PODLOGA I NUTRIENT SUPPLEMENT MEDIUMA

Sve one serije koje su prošle kontrolu kvaliteta, odobravaju se za dalji process prerade i proizvodnje, koji podrazumeva presipanje pune krvi u finalnu ambalažu za krajnje korisnike ili upotrebu pune krvi za izlivanje podloga u petri šoljama.

7) PAKOVANJE I OTPREMA PROIZVODA

Nakon ponovne kontrole, serije gotovih podloga pakuju se u finalnu ambalažu i skladište u magacinu, do isporuke korisniku.

U svim našim prostorijama i magacinima vrši se konstantan monitoring ambijentalnih uslova, pre svega temperature i vlage.

8) TRANSPORT PROIZVODA DO KUPCA POD TEMPERATURNIM REŽIMOM

Kada dođe vreme za distribuciju, podloge i krvni proizvodi se pakuju u transportne kutije i našim specijalizovanim vozilima, sa dva temperaturna režima i data logerima, koji prate i ispisuju kretanje temperature u toku transporta, isporučuju se krajnjem korisniku.

9) ZADOVOLJNI KORISNICI – UPOTREBA PROIZVODA U LABORATORIJI

Svaki naš korisnik, uz isporuku robe koja zahteva hladni temperaturni režim, dobija dokaz o kretanju temperature u toku transporta, kao i sve potrebne sertifikate kontrole svake isporučene serije, a standardi koje smo uveli i obnavljamo ih svake godine, garancija su kvaliteta proizvoda i njegovog čuvanja.

Sve to zajedno, čini korisnika spokojnim i bezbrižnim u daljem radu sa našim proizvodom.

Slika 1. Dobijanje sirovina, proizvodnja, kontrola kvaliteta i transporta ProReady proizvoda

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OPTIMIZACIJA FLEBOTOMIJE I POZNAVANJE ERITROPOEZE U CILJU DOBIJANJA KVALITETNE KRVI KAO PRIMARNE SIROVINE

Flebotomija predstavlja postupak uzimanja krvi u dijagnostičke ili terapijske svrhe tako što se vrši plasiranje ugle u neku anatomski dostupnu venu. Krv ima veliki potencijal samoobnavljanja, ali postoji potreba da se standardizuje način i zapremina uzete krvi, kako se čestim flebotomija ne bi životinja uvela u hipovolemiju ili anemiju. Krv se uzima u određenoj zapremini, a određeni su standardi za različite životinjske vrste. Radi se o onoj zapremini krvi koja se može uzeti, a koja neće izazvati određena bolesna stanja kod jedinke niti jatrogenu anemiju ili hipovolemiju. Zapremine su prikazane u tabeli i odnose se na zdrave, za vrstu uobičajene, norlamne i odrasle jedinke. Ukoliko se radi o bolesnim životinjam mlađim životinjama ili životinjama koje su podvrgnute nekom eksperimentu visoke invazivnosti ove zapremine moraju biti redukovane.

Ukupna zapremina krvi u cirkulaciji je 5,5-8% telesne težine životinje. Uzimanje krvi koje ne podrazumeva dodatno praćenje i koje ne može da dovede do nekog terminalnog nepovoljnog ishoda je 10% ukupne zapremine krvi. Smatra se da prikupljanje krvi u ovoj zapremini može biti ponavljano na dve nedelje kod longotudinalnih, serijskih uzimanja, kao što se to često čini kod ovnova čija se krv koristi za biotehnološke procese (npr.kod proizvodnje mikrobioloških podloga ili kod proizvodnje krvi za istraživačke svrhe. Tako se kod ovnova može uzeti 6,6 ml/kg krvi na svake dve nedelje. Ukoliko se koriste ovnovi koji imaju oko 60 kg, to znači da se maksimalno može uzeti 396 mL krvi u jednom vađenju u dve nedelje. Uzimanje veće zapremine preko ove mora biti pod nadzorom i preporučuje se davanje određene zapremine tečnosti radi nadoknade. Odmor između dva vađenja krvi mora biti optimalan. Ukoliko se uzme oko 1% krvi sledeće vađenje se može ponoviti već narednog dana, kod uzimanja 5-7% krvi sledeće uzimanje se može vršiti za nedelju dana, ako se uzme oko 10% krvi onda period odmora do sledećeg vađenja minimum 2 nedelje, a ako se uzme 15% zapremine cirkulišuće krvi onda odmor mora trajati minimalno mesec dana, odnosno 4 nedelje.

Tabela 1. Količina krvi u organizmu i maksimalno dozvoljena zapremina uzete krvi flebotomijom

Vrsta	Zapremina krvi (mL/kg)		Pojedinačno uzimanje krvi - maksimalno 10% zapremine krvi (mL/kg)
	Vrednost	Opseg	
Ovce	66	66 (60-74)	6.6
Goveda	55	55	5.5
Koze	66	--	6.6
Svinje	65	65 (61-68)	6.5
Psi	86	86 (79-90)	8.6
Mačke	55	55	5.5

Uzorkovanje krvi se vrši iz različitih dostupnih krvnih sudova, a kod ovnova je najpovoljnija jugularna vena. Uzimanje krvi iz ove vene je relativno jednostavno i ono omogućuje dobijanje srednje do velike zapremine krvi. Uzorci dobijeni iz vene jugularis su dobrog do odličnog kvaliteta. Uzimanje krvi iz ove vene ne zahteva anesteziološki protokol, ali su potrebne mere asepse i antisepse kako bi se dobila kvalitetna i održiva krv. Višekratno uzimanje krvi iz jugularne vene može biti izvor problema, pa je potrebno da se vena, odnosno strana menja, te da se izbegavaju višekratna uzorkovanja u kratkom vremenskom intervalu iz jugularne vene.

Prilikom kontinuiranog uzimanja krvi potrebno je vršiti monitoring životinje. Monitoring podrazumeva ispitivanje hematokrita i zapremine eritrocita, da bi se ustanovilo da li životinja ima potencijal da održava stalnost zapremine eritrocita i da svaki eritrocit ima adekvatnu zapreminu, odnosno da nema razvoja mikrocitoze zbog gubitka hemoglobina i gvožđa. Zdrave, odrasle životinje mogu u okviru 24 sata da povrate zapreminu tečnosti uzete krvi, ali je za vraćanje hematokrita na adekvatan nivo i dolazak novih eritrocita potrebno vreme, pa se mora optimizirati vreme između dva vađenja krvi. Akutni gubitak veće

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količine krvi neće odmah pokazati promene u crvenoj lozi ili hematokritu, jer se pokreće stresna reakcija i deluju vazokonstroktori kako bi se očuvao punjenost i turgor krvnih sudova. Zbog toga ove kontrole treba sprovesti tek po isteku 24-48 sati posle poslednjeg vađenaj krvi. Smatra se da ukoliko je hematokrit ispod 35%, a koncentracija hemoglobina manja od 100g/L ne sme se pristupiti uzimanju krvi od takve životinje. Svakako potrebno je poznavati referentne i normalne vrednosti (koje su uvek nešto šire od referentnih) za vrednosti hemoglobina i hematokrita kako bi se procenilo da li se krv može uzeti, u kojim vremenskim intervalima i kojoj pojedinačnoj i ukupnoj zapremini. Ukoliko je životinja mlada ili se radi o uzimanju veće količine krvi kada je životinja u riziku od dehidracije ili prerenalne azotemije onda se posle uzomanja zapremine krvi može dodati sterilni, izotonični fiziološki rastvor, a pored njega dobro je dati rastvor Ringer-laktat.

Koje su posledice flebotomije i gubitka krvi kod životinja? U jednom starijem ogledu ispitan je redosled karakterističnih promena progresivnog deficita gvožđa pokazan je serijskim krvarenjem normalnih dobrovoljaca i pacijenata sa policitemijom. Radi se o ogledu sprovedenog na ljudima. Posle puštanja krvi u perifernoj krvi su se javile promene po sledećem redosledu: a) pad koncentracije hemoglobina; b) smanjeno gvožđe u plazmi; c) retikulocitoza, povećanje MCV i MCH; d) smanjenje MCV i MCH, povećanje ukupnog proteina koji vezuje gvožđe; i e) smanjen MCHC. Karakteristične promene nedostatka gvožđa su se vratile na nivoe pre flebotomije u sledećem redosledu: a) koncentracija hemoglobina; b) ćelijski indeksi; c) serumsko gvožđe; g) protein koji vezuje gvožđe u serumu; i e) hemosiderin koštane srži, i na kraju se povećana gastrointestinalna apsorpcija gvožđa vratila u normalu. Ubrzana proizvodnja crvenih krvnih zrnaca nastavljena je kod pacijenata sa policitemijom uprkos indukciji umerenog nedostatka gvožđa. Kvalitet je žrtvovan za kvantitet, i na taj način je došlo do dublje mikrocitoze nego kod normalnih subjekata sa sličnim stepenom nedostatka gvožđa.

Eritron je anatomsko-funkcionalna jedinica eritropoeze i čini kompletnu ćelijsku populaciju od progenitora opredeljenih za eritrocitnu lozu, do zrelih eritrocita koji cirkulišu u krvi.U meduli eritrona se nalaze ćelije opredeljeni progenitori (BFU-E –Burst Forming Unit Erythroid i CFU-E-Colony Forming Unit Erythroid) i prekursori, u koje se ubrajaju različite vrste eritroblasta, koje su morfološki prepoznatljive ćelije eritrocitne loze u aktivnoj proliferaciji.Eritrocitni progenitori čine celinu u kojoj se BFU-E se diferentuju i stvaraju se CFU-E. Za proces eritrocitopoeze je neophodno i gvožđe, kao sastojak molekula hema, koji je sastavni deo hemoglobina (Hb). Gvožđe kao sastavni deo molekula hema, dolazi preko proteina transferina, čiji se receptori nalaze u malom broju ćelija BFU-E. Broj receptora za transferin, raste na CFU-E i eritrocitnim prekursorima odnosno eritroblastima u različitim stadijumima zrelosti, a kod retikulocita se smanjuje. Pošto su Erci dominantne ćelije krvi,njihova homeostaza zahteva veliku rezervu prekursora pa zato na eritroblaste otpada četvrtina ukupnog sadržaja kosne srži odraslih životinja.Nakon prelaska koncentracije Hb iznad 80% u eritroblastu (acidofilnom), on prestaje da proliferiše, kroz kapilarne sinuse endotela kosne srži prelazi u krv, izbacuje jedro i tada nastaje retikulocit. Proces sazrevanja retikulocita u eritrocite u cirkulaciji ili slezini traje između 48-96 sati.Na osnovu njihovog prisustva u cirkulaciji procenjuje se eritropoetska aktivnost kostne srži, jer se oni kod većine vrsta oslobađaju u cirkulaciju posle ubrzane hemolize i hipoksičnih stanja.Broj retikulocita u krvi zavisi od dužine života Erci.U fiziološkim uslovima retikulociti su u zavisnosti od vrste životinje zasupljeni 0-2%. Kod konja se Erci iz kostne srži otpuštaju u zrelom obliku, tako da ih nema u cirkulaciji. U uslovima ubrzane eritropoeze, posle akutnog gubitka krvi ili hemolitičkih anemija, retikulociti su odsutni ili su veoma redak nalaz u cirkulaciji.Zato se kod konja punkcijom kostne srži utvrđuje regenerativna anemija. Kod velikih preživara retikulociti su u fiziološkim uslovima redak nalaz u perifernoj krvi ali kod akutnih ,masivnih gubitaka krvi ili masivnog krvarenja dolazi do povećanja njihovog broja. Kod ovaca i koza retikulociti se u fiziološkim uslovima retko nalaze u perifernoj krvi ali im se posle akutnih krvarenja, broj povećava do 6%. Proces eritropoeze u kome dolazi do stvaranja zrelih Erci traje 7-8 dana.Kod pasa, mačaka, svinja i goveda u toku fiziološke eritropoeze, dnevno se proizvodi $6x10^{11}$ erci, koji sadrže oko 7g Hb, međutim ukoliko dođe do masovnog krvarenja kod životinja, može se proizvesti i do 6 puta više eritrocita. Kod ovaca,koza i konja u slučajevima potreba, vrednosti proizvedenih eritrocita mogu da budu i dvostruko više. Ukoliko dođe do procesa ubrzane eritropoeza, tada ee broj retikulocita u poređenju sa fiziološkim vrednostima retikulocita znatno povećava, kako u cirkulaciji tako i u kostnoj srži a njihov broj zavisi i od vrste životinja kod kojih se proces ubzane eritropoeze javlja. Kod anemija retikulocitoza ukazuje na gubitak krvi ili povećanu destrukciju eritrocita.Ukoliko ne postoji anemija,

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pojava retikuloctoze ukazuje na smanjenu oksigenaciju krvi, ili na smanjenu tkivnu perfuziju. Zbog smanjene oksigenacije dolazi do hipoksije, koja povećava sekreciju eritropoetina, koji stimuliše eritropoezu. Kod pacijenata sa anemijom,postoji odsustvo retikuloze te dolazi do smanjenja sinteze eritropoetina, javlja se depresija kostne srži, dolazi do nedovoljnog korišćenja gvožđa te se javlja povećana, a praktično neefikasna eritropoeza. Pri postojanju fiziološke ravnoteže efikasnost eritrocitopoeze je oko 85-90%, što znači da oko 10-15% ćelija ne dostiže zrelost zbog greške u stvaranju, te dolazi domiranja ćelija. U slučaju anemija ovaj deo ćelija koji ne dostižu zrelost u neefikasnoj eritropoezi, se povećava.Tako kod eritropoetske porfirije goveda i trovanja olovom kod pasa nalazimo navedenu pojavu. Ćelije koje nastaju u pri povećanju neefikasneeritropoezekraće ili duže vreme ostaju u kostnoj srži te sestvara utisak „hiperplazije“ crvene krvne loze. Kontinuirano uzimanje krvi dovodi do deficita gvožđa i hemoglobina

INTERNA KONTROLNA LABORATORIJA

Interna kontrolna laboratorija, tj. kontrola kvaliteta Proready proizvoda otvorena je 2019. godine. Prilagođena je specifičnim potrebama kontrole i razvoja široke palete gotovih mikrobioloških podloga koje se koriste u kliničkoj i sanitarnoj mikrobiologiji, kao i u farmaceutskoj industriji. Kao takva, u infrastrukturnom, metodološkom i kadrovskom smislu, neophodno je da zadovoljava visoke standarde DLP (GLP) i DPP (GMP)-ja.

U osnovnom smislu, aktivnosti u Proready laboratoriji možemo podeliti na nekoliko celina:

1) ULAZNA KONTROLA SIROVINE

Kako bi bili sigurni u predvidljivost rezultata i stalnost kvaliteta svake serije gotovih podloga, neophodno je da se, pored procesa i opreme, kontroliše i svaki sastojak pri nabavci i ulazu. Dehidratisane sirovine, suplementi u suvoj i tečnoj fazi, krvni sastojci, ambalaža i osnovni sastojak – demineralizovana voda, sve to podleže kontroli. Deo sastojaka se kontroliše kroz proveru ulazne dokumentacije i sertifikata, pa onda, proverom ključnih performansi kroz testove i izradu i kontrolu malih probnih serija. Pored toga, puna ovčija i konjska krv, kao suplementi podloga, u ovoj fazi se kontrolišu i potvrđuje se sterilnost svake serije (mikrobiološka čistoća). Pre svake serije, demi voda koja ulazi u sastav podloge, takođe se testira kroz proveru relevantnih parametara (provodljivost, boja, pH…).

2) AMBIJENTALNI MONITORING I INPROCESNA KONTROLA

Pošto se izlivanje podloga odvija u aseptičnim uslovima, tj. čistim sobama određene klase, a i sama laboratorija se nalazi u klasiranom prostoru (čista soba klase ISO 8), svakodnevno se vrši mirkobiološki monitoring vazduha, površina, rukavica i odela operatera kako u proizvodnji tako i u laboratoriji.

Nakon uzorkovanja, obrada uzoraka (inkubacija i očitavanje) se vrše u kontrolnoj laboratoriji i prati se trend rezultata.

3a) KONTROLA MIKROBIOLOŠKIH PERFORMANSI SERIJE GOTOVOG PROIZVODA

Nakon proizvodnje svake serije uzorkuje se reprezentativan broj uzoraka i u laborotoriji se radi kontrola mikrobioloških performansi, što je ključno.

Iako sam sistem kvaliteta, procedure i primena standarda, osigurava stalnost kvaliteta, svaka serija se kontroliše bez izuzetka.

Kontrola podrazumeva zasejavanje ploča ATCC sojevima mikroorganizama, u zavisnosti od standarda i namene podloge (klinika, sanitarna bakteriologija ili farmacija) tj. u skladu sa odgovarajućom metodom.

Proverava se produktivnost, selektivnosti i/ili specifičnost.

Svi rezultati se upisuju u interna dokumenta, a zatim se izradjuje sertifikat.

3b) KONTROLA MIKROBIOLOŠKE ČISTOĆE SERIJE GOTOVOG PROIZVODA

Da bi neka podloga mogla da se koristi shodno nameni, mora zadovoljniti kriterijume odgovarajuće mikrobiološke čistoće, što najčešće podrazumeva sterilnost. Sam proces kuvanja, sterilizacije i aseptično nalivanje podloga u svim oblicima (ploče, epruvete i boce) obezbedjuje zahtevanu čistoću, ali se bez izuzetka, od svake serije uzima reprezentativan uzorak i inkubira na dva temperaturna režima, kako bi se potvrdio izostanak rasta bakterija i gljiva. Rezultati testiranja svake serije se upisuju u sertifikat.

3c) KONTROLA FIZIČKO HEMIJSKIH PERFORMASI I VIZUELNA KONTROLA

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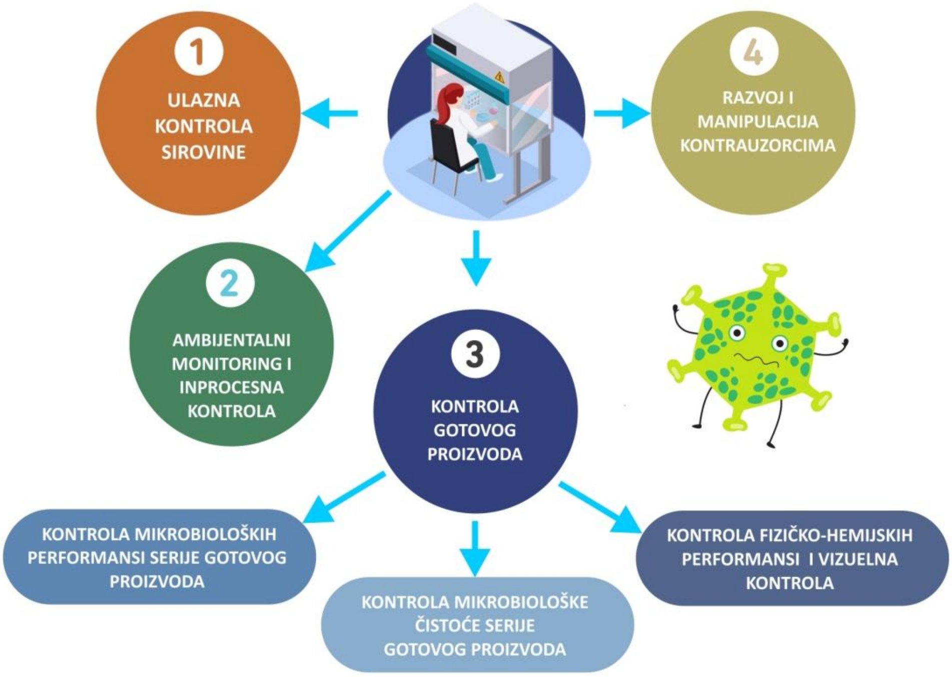
Pored navedenih kontrola, potrebno je da pH vrednost bude u određenom opsegu u skladu sa zahtevima, što se proverava specijalnim sondama za merenje pH sa površine same podloge, ako je u pitanju petri šolja. Na kraju, proverava se prozirnost podloge, adekvatna boja i debljina sloja nalivanja. Deo ovih kontrola se odvija inprocesno, u samom pogonu, a takođe se sve analize ponovo rade na gotovom proizvodu u samoj laboratoriji.

4) RAZVOJ I MANIPULACIJA SA KONTRAUZORCIMA

U nameri da našim korisnicima rad učinimo lakšim i pouzdanijim, u sektoru za razvoj neprestano radimo na usavršavanju naših proizvoda, tamo gde je to moguće. Pre svega se radi o povećanju stabilnosti, produžavanju roka trajanja, uvođenju novih veličina ili oblika pakovanja. U ovom procesu, laboratorija ima značajnu ulogu, pošto sam razvoj zahteva sprovođenje velikog broja testova, probnih serija, ogleda i posmatranja. Informacije, rezultate i zapažanja, do kojih dolazi laboratorija, veoma su bitni u nastojanju da se dođe do nekog pomaka. Od svake proizvedene serije se po pravilu čuva deo uzoraka i to sve do isteka roka trajanja. Evidenciju svih ovih uzoraka vodi laboratorija.



Slika 2.Interna kontrola kvaliteta prema ProReady standardima

OPREMA

ProMedia je svoju proizvodnju opremila po vodećim svetskim standardima, diktiranim od strane farmaceutskih kuća, a vođena dobrom praksom i pozitivnim primerima svog mentora, kompanije HiMedia Laboratories.

Linija odmeravanja i pripreme strogo je kontrolisana, kako u pogledu ispravnosti i preciznosti merila, tako i u pogledu zaštite i bezbednosti zaposlenih. Ovde smo sproveli najstrožije svetske standarde, s'obzirom na činjenicu da zdravlje i bezbednost na radu naših zaposlenih nema cenu.

Linija pripreme hranljivih medijuma odvija se sinhrono u tri mediapreparatora, dok je automatsko punjenje u kontrolisanom prostoru Klase ISO7, sa čistim tunelom Klase ISO 4.

Sve ovo garantuje visok stepen pouzdanosti naših proizvoda i stabilnost u pogledu konstantnosti i ujednačenosti u kvalitetu hranljivih podloga.

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Zastupljena je sledeća oprema:

- Čista soba klase ISO 7
- Tunel za izlivanje klase ISO 5
- Preko 100 kvadrata kontrolisanog prostora
- 24h/7 nadzor i zapis ambijentalnih uslova (pritisak, temperatura, vlaga)
- Automatizovana proizvodnja (2000 ploča/sat), veličina serije do 10000 kom
- Punilica- IQ design USA, Mediapreparatori- Systec, A.B.E., Pakerica – IQ design USA

SISTEM KVALITETA

Zvanična potvrda za ulazak u svet kvaliteta je sertifikat koji je kompanija ProMedia dobila za međunarodni standard ISO 9001 pre gotovo dvadeset godina, 2006.godine.

Uvođenjem standarda ISO 14001 i ISO 18001, 2014. godine, dobili smo još jednu potvrdu kvaliteta našeg dugogodišnjeg rada.

Sistem upravljanja kvalitetom se efikasno sprovodi i u proizvodnji i od 2017-te godine, sertifikovani smo i po ISO13485 standardu, za proizvodnju medicinski sredstava.

Kvalitet proizvoda je prioritet naše proizvodnje i postignut je kroz standardizaciju svih faza proizvodnog procesa i testiranjem kvaliteta svake proizvedene serije.

Proizvodne i operativne procedure, zapisi i svi procesi vezani za proizvodnju i kontrolu postavljeni su u skladu sa smernicama dobre proizvođačke prakse (GMP).

ProReady Interna kontrolna laboratorija bavi se kontrolom ulazne sirovine i svake proizvedene serije, praćenjem fizičko-hemijskih i mikrobioloških preformansi, kao i ambijentalnim monitoringom.

Naš tim visoko obrazovanih i iskusnih stručnjaka u kontroli kvaliteta kontroliše i garantuje kvalitet svake proizvedene serije, prateći i primenjujući metode najznačajnijih internacionalnih standarda.

Ove i druge korisne informacije i pojedinosti i proizvodnim kapacitetima i ponudi kompanije ProMedia i o programu ProReady možete naći na sledećim stranicama: https://www.promedia.rs/; https://www.proready.rs/.

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RADIONICA:
BIOSIGURNOSNE PRAKSE NA FARMAMA PREŽIVARA I KAKO KOMUNICIRATI SA FARMERIMA

Slavča Hristov, Branislav Stanković, Milica Rađenović*

*Univerzitet u Beogradu – Poljoprivredni fakultet, Beograd-Zemun, Republika Srbija

S.H. https://orcid.org/0000-0002-1719-9971, B.S. https://orcid.org/0000-0003-3925-6102

Struktura radionice
Polazna tačka: 1. da li obično razgovarate o preventivnim merama (biosigurnosnim merama) sa farmerima vezano za određenu bolest? i 2. da li ste upoznati sa bilo kakvim programom poboljšanja biosigurnosti na farama u zemlji, u vezi sa nekom specifičnom bolešću?

Teme za diskusiju na radionici:
Sat 1: Izazovi za implementaciju biosigurnosti na farmama
Sat 2: Šta je potrebno za poboljšanje biosigurnosti na farmama preživara u regionu?
Sat 3: Koje bi bile najadekvatnije strategije za poboljšanje komunikacije?

U okviru diskusije u vezi sa prvim dvema temama, pripremljene su liste pitanja o kojima će se raspravljati na radionici na osnovu literature i iskustva autora.
Lista pitanja je raspoređena u nekoliko grupa i to: vezano za veterinare, za farmere, za vrste proizvodnje, za farme i pašnjake, za posetioce, za saradnju između zainteresovanih strana, za kompanije i izvođače, za na socioekonomske faktore, koji se odnose na kreatore politike, i na druge faktore.
Ova grupa pitanja će pomoći u razgovorima sa učesnicima na radionici.

SPISAK PITANJA KOJA ĆE SE RAZMATRATI NA RADIONICI

Sat 1: Izazovi za implementaciju biosigurnosti na farmama:
A. vezano za veterinare: 1. nedostatak odgovarajućeg obrazovanja za veterinare, nivo percepcije veterinara o biosigurnosti može varirati, mnogi od njih nisu imali adekvatnu obuku nakon diplomiranja, 2. višestruke posete farmi od strane veterinara (vozilo, oprema, ponašanje osoblja u praksi), 3. mnogi veterinari ne izdvajaju vreme da razgovaraju sa farmerima o planovima za biosigurnost, 4. veterinarima se ne postavljaju pitanja u vezi sa biosigurnošču farme, stoga veterinari pretpostavljaju da se protokoli sprovode ili često smatraju da nisu kvalifikovani da daju savete, 5 nedostatak jasnih informacija o biosigurnosti, 6. nedovoljni dokazi o efikasnosti biosigurnosnih praksi, farmera i drugih stručnjaka za zdravlje stada, 7. nedostatak tačnih informacija i kvantitativnih podataka koji istinski povezuju poboljšanje biosigurnosti, zdravlja i proizvodnje životinja, 8. nejasne informacije o obaveznim testovima pri kupovini životinja, 9. nepostojanje precizne definicije biosigurnosnih mera i nerazumevanje razlike između nhihovog značaja i efikasnosti, 10. percepcija manje opasnosti od bolesti u zavisnosti od istorijijata pojave bolesti u regionu i finansijskih ograničenja, 11 nedostatak znanja o efikasnosti biosigurnosti, ali i o poboljšanju biosigurnosti u odnosu na potencijalne ulazne tačke bolesti, itd.
B. koja se odnose na farmere: 1. nedostatak razumevanja o načinima prenošenja bolesti, 2. neadekvatno znanje o putevima infekcije, nedostatak obrazovanja za promenu stavova, 3. nedostatak obuke: ne pruža se formalna obuka za novo osoblje na farmama, 4. nedostatak finansijskih sredstava, 5. psihološke barijere i drugi zbunjujući faktori, 6. troškovi (u vremenu i novcu), 7. sposobnost i volja farmera da investiraju u mere biosigurnosti (glavno ograničenje koje vide veterinari), 8. nekorišćenje društvenih mreža ili struktura zajednica farmera (udruženja) u smislu stavova i motivatora za sprovođenje mera prevencije bolesti, 9. emocionalno stanje i percepcije farmera, 10. dodatna radna snaga, očekivano potrebno ulaganje u rad, vreme

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i kapital, više administracije (registracija posetilaca), dodatna pravila i inspekcije, 11. nejasne informacije o obaveznim ispitivanjima pri nabavci životinja, troškovima protokola pri kupovini životinja i ignorisanje, 12. farmeri nisu svesni troškova i gubitka prihoda zbog bolesti životinja, 13. različita percepcija rizika, 14. percepcija efikasnosti i nerazumevanje biosigurnosnih praksi, 15. farmeri imaju malo vere u efikasnost mera biosigurnosti na farmama u odsustvu akcija drugih, kao što je Vlade koja obezbeđuje granice - da spreči rizik usled ilegalnog uvoza, 16. nedostatak objekata na farmama, nedostatak saradnje farmera, 17. percepcija farmera koristi od mera i zdravstvene odgovornosti, kao i niskog rizika, 18. psiho-socio-demografski faktori utiču na ponašanje farmera u smislu primene biosigurnosti, 19. beskorisnost po percepciji farmera, zbog odsustva problema, 20. farmeri često ne sprovode svoje mere kontrole i oslanjaju se na profesionalizam posetilaca i organizatora događaja, 21. spremnost pojedinaca za promene i uverenja da su bolesti neizbežne utiču na nivo biosigurnosti farme, 22. intervencije biosigurnosti namenjene malim zemljoposednicima nisu „jedna veličina za sve“, stoga unapred definisane intervencije često ne funkcionišu kako je planirano, 23. nedostatak znanja o efikasnosti biosigurnosti, ali i o poboljšanju biosigurnosti u pogledu potencijalnih ulaznih tačaka bolesti predstavlja izazov, itd.

C. koji se odnose na tip proizvodnje: 1. uzgoj mešovitih vrsta može predstavljati izazov za biosigurnost na farmi životinja, 2. otvoreni sistem uzgoja, veličinu stada, 3. visok stepen mešanja životinja sa različitih farmi, 4. proizvodnja mleka u stadu kao biosigurnosni rizik, 5. organski status kao biosigurnosni rizik, 6. povećana veličina stada bez adaptacije objekata za smeštaj stoke, 7. hobi ili mali proizvođači imaju nizak nivo biosigurnosti na farmama, 9. favorizuju se različite mere biosigurnosti na velikim i malim farme, a manje se primenjuju na manjim farmama, 10. najniži nivoi biosigurnosti su povezani sa mešovitim farmama, 10. farmeri pogrešno shvataju koncept 'zatvorenog stada', itd.

D. vezano za farme i pašnjake: 1. blizina farmi, 2. geografski region, 3. tip držanja i smeštaja, 4. nedostatak prostora za održavanje fizički odvojenog prostora, 5. pozajmljivanje opreme, 6. infrastruktura farme, raspoloživi prostor i uticaj tipa proizvodnje na kompartmentacija, 7. gubljenje prostora i dodatno radno vreme potrebno za održavanje koridora kod gajenja bez ispaše, 8. mogućnost sprečavanja kontakta sa divljim životinjama, 9. tradicionalni sistem zakupa zemljišta i visoka zastupljenost farmi u neposrednoj okolini imaju negativan uticaj na biosigurnost farmi, itd.

E. vezano za posetioce: 1. veliki broj posetilaca, 2. veliki broj poseta, 3. različiti posetioci imaju različite uslove za održavanje biosigurnosti na farmama, 4. mnogi posetioci su prijavili da nemaju pristup u objektima uređajima za pranje ruku itd.

F. u vezi sa saradnjom između zainteresovanih strana: 1. farmeri imaju malo poverenja u efikasnost mera biosigurnosti na farmama u odsustvu akcija drugih, kao što je Vlada koja obezbeđuje granice za sprečavanje ilegalnog uvoza, 2. farmeri, veterinari i pomoćne industrije - nedostatak poverenja u međusobnu implementaciju biosigurnosti, 3. odnos između farmera i veterinara, društvena dinamika, raspoloživo vreme i prostor, radnici na farmama koji nemaju odgovarajuću obuku i nepoverenje prema javnoj upravi, 4. neusaglašene poruke i preporuke, negativna percepcija kontrolnih organa, nedostatak kontrole na drugim farmama/profesionalni posetioci/divljač, nepostojanje konsolidacije zakonskih zahteva, kontradikcija u merama između sektora, više ograničenja, povećani troškovi proizvodnje, nekonkurentne cene itd.

G. koja se odnose na kompanije i izvođače radova: 1. kontakti na nivou farme sa kompanijama i izvođačima koji ne sprovode preventivne prakse biosigurnosti, 2. kompanije za tovne junadi - nedostatak informacija o poboljšanju upravljanja farmama i implementaciji biosigurnosnih mera, 3. poteškoće u komunikaciji između farmera, njihovih zaposlenih i posetioca, zbog problema kao što su različiti jezici i dijalekti, 4. uloga kupaca mleka i veterinara je važna u primeni svih vrsta biosigurnosnih mera itd.

H. u vezi sa socioekonomskim faktorima: 1. neujednačene prakse biosigurnosti na farmama se različito razmatraju između društvenih grupa, geografskih udaljenosti i lanaca poljoprivrednih proizvoda, 2. ruralno siromaštvo, 3. mnogi ruralni veterinari su prijavili retko dostupnu infrastrukturu za biosigurnost na farmama, kao što su prostori za teljenje ili izolacije, itd.

I. koji se odnose na kreatore politike: 1. nedostatak zakona i propisa, 2. čekanje smernica koje treba da slede, 4. višak zakona, birokratije i potencijalni gubitak autonomije farmera, 5. neadekvatna kontrola granica, 6. neefikasne politike i regulative, 7. nezainteresovanost za implementaciju biosigurnosnih mera na farmi vezuje se za kolektivnu atribuciju, 8. postoje jaki negativni stavovi prema određenim izvorima informacija itd.

J. u vezi sa drugim faktorima: 1. velika udaljenost od veterinarskih službi može predstavljati problem, 2. postoji nedostatak čvrstih dokaza koji su potrebni za preporuke o vrstama biosigurnosnih intervencija za smanjenje prenošenja bakterijskih infekcija sa životinja na ljude, odnosno koje su najefikasniji za poboljšanje ishoda zdravlja ljudi, 3. nepredviđene prepreke itd.

Sat 2: Šta je potrebno za poboljšanje biosigurnosti na farmama preživara u regionu?

A. koji se odnose na veterinare: 1. pružanje pristupačnih i praktičnih informacija o biosigurnosti, 2. informacije o jednostavnim merama biosigurnosti i upornoj podršci i bolje razumevanje troškova i gubitaka prihoda uzrokovanih bolestima, 3. menjanje percepcije efikasnosti kroz uključivanje veterinara u diskusije o biosigurnosti na farmi može dovesti do usvajanja biosigurnosnih praksi, 4. poboljšati formalno zdravstveno planiranje je rešenje za promociju biosigurnosti, 5. poboljšati komunikacijske veštine veterinara kako bi uticali na farmere, 6. poboljšati diskusije između veterinara i farmera, 7. baviti se stvarnim infektivnim rizicima, izvodljivošću i ekonomičnošću kada se preporučuju mere biosigurnosti, 8. važno je naglasiti stvarne koristi zasnovane na dokazima, 9. različitim mentalnim konstruktima treba se baviti komunikacijskim strategijama, 10, rešavanje prioritetnih pitanja je ključna strategija za donošenje mera biosigurnosti, 11. neophodno je identifikovati najvažnije bolesti koje pogađaju farme životinja kako bi se mogle uvesti specifične mere koje se odnose na javno-zdravstvene svrhe, 12. biosigurnost mora biti više uključena u veterinarke programe obuke jer veterinari treba da imaju aktivniju ulogu u smislu usmeravanja i poboljšanja implementacije biosigurnosti na nivou farme, 13. ruralnim veterinarima su potrebne odgovarajuće smernice od strane vlasti, odgovarajuća obuka o biosigurnosti i komunikaciji, kao i okruženje koje to omogućava, 14. poznavanje veterinarske epidemiologije i razumevanje prenošenja i širenja bolesti, faktora rizika i metoda prevencije bolesti važni su za razvoj programa kontrole bolesti i sprovođenje programa biosigurnosti na farmama, regionalnom i nacionalnom nivou, 15. postoji potreba da se kreiraju preventivne strategije specifične za farmu u saradnji sa veterinarima, 16. planiranje vanrednih situacija u vezi sa pojavom bolesti u nastajanju ili suzbijanje endemskih bolesti može se olakšati korišćenjem informacija o tome koje mere biosigurnosti treba da se koriste, 17. treba obezbediti obuku uz veće učešće veterinara koja treba da bude prilagođena definisanim grupama i specifičnim potrebama, uz korišćenje novih tehnologija i sistema za evidentiranje, 18. po mogućnosti obuka treba da bude u direktnom format ("licem u lice"), 19. prilagođavanje strategija za širenje informacija, unapređenje pristupa veterinarskim uslugama i davanje prioriteta obrazovanju u ključnim oblastima, 20. bliže usklađivanje komunikacijskih strategija sa preferencijama ciljne publike može maksimizirati usvajanje informacija o biosigurnosti, 20. aktivan dijalog sa farmerima se preporučuje za bolju implementaciju biosigurnosti, 21. važno je pružiti pomoć u razvoju, angažovate male farmere koji su voljni da poboljšaju sisteme, dozvoliti skeptičnim malim vlasnicima da posmatraju i učestvuju u trenutku kada im odgovara i povezati biosigurnost sa dobicima kao što su prihod i ušteda vremena itd.

B. koji se odnose na farmere: 1. povećati svest – bolja komunikacija, 2. više obuke, 3. više resursa za poboljšanje infrastrukture, 4. obuka u izradi planova biosigurnosti, 5. edukacija farmera treba da se unapredi kroz obuku koja bi istakla značaj praksi biosigurnosti, 6. poboljšati prihvatanje i sprovođenje praksi biosigurnosti kroz poboljšanje obrazovanja farmera, 7. programe obuke za promenu stavova i percepcije proizvođača o lošim praksama biosigurnosti, 8. ograničavanje nabavke životinja na farmama, 9. postoji potreba za unapređenjem znanja farmera o zdravlju i proizvodnji preživara, 10. primenjeno istraživanje na terenu, obuka "na poslu" i "formalni" programi obuke daju potrebne informacije farmerima, 11. dokumentovanje implementacije biosigurnosti na farmi može biti korisno za zemlje koje se suočavaju sa proširenjem farmi, 12. treba obezbediti bolji pristup farmerima izvorima informacija i saveta o biosigurnosti, 13. važno je pružiti razvojnu pomoć, angažovati male farmere koji su voljni da poboljšaju sisteme, dozvoliti skeptičnim malim farmerima da posmatraju i učestvuju uzimajući u obzir njihovo pogodno vreme i povezivanje biosigurnosti sa dobicima kao što su prihod, ušteda vremena itd.

C. koje se odnose na tipove proizvodnje: 1. mere biosigurnosti zasnovane na individualnim karakteristikama farme mogle bi da pomognu u poboljšanju biosigurnosti farme, 2. manje farme treba da poboljšaju nivo biosigurnosti, stoga se preporučuje savetovanje u vezi sa potencijalnim rizicima itd.

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D. u vezi sa farmama: 1. identifikovati šta je izvodljivo na farmama, 2. treba uvesti programe biosigurnosti na nivou sela, 3. koristiti pravilno opremu na farmama, 4. nivo biosigurnosti farme je veoma važan pokazatelj potencijalnog poboljšanja, stoga ga treba proceninjivati, 6. treba posvetiti veću pažnju sprečavanju direktnog kontakta sa životinjama iz drugih stada itd.
E. u vezi sa saradnjom između zainteresovanih strana: 1. saradničke akcije i preuzimanje odgovornosti među svim operaterima u industriji treba da budu promovisane od strane javnog sektora, 2. poboljšani alati za podršku odlučivanju koji će prednosti poboljšane biosigurnosti farme učiniti očiglednijim, 3. identifikovanje komunikacijskog jaza između istraživanja i industrije i rešavanje istog u vidu obuka za veterinare, 4. uspostavljanje dobrih kanala komunikacije između farmera, veterinara i drugih savetnika za zdravlje stada je neophodno, 5. promovisanje edukacije o zaraznim bolestima i merama prevencije bolesti među farmerma, 6. obuka treba da bude participativna i treba da uključi zajednice, ali i da ohrabri učesnike da prevaziđu praktične i kulturne prepreke u cilju poboljšanja nivoa biosigurnosti na farmi, 8. potreban je novi pristup za stvaranje kolektivnog ponašanja u smislu implementacije biosigurnosti, stoga neutralna komunikacija treba da bude ohrabrena itd.
F. koji se odnose na kompanije i ugovarače: 1. distribucija proizvoda za čišćenje i dezinfekciju od strane kompanija, 2. različite zainteresovane strane treba da preduzmu mere u vezi sa merama biosigurnosti, 3. vlasnici, menadžeri i radnici u stočarskim preduzećima, industrijskim telima i ruralne i urbane zajednice treba da budu uključene u obuku u vezi sa biosigurnošću, 4. treba koristiti multidisciplinarni pristup za rešavanje pitanja poboljšanja biosigurnostii na farmi uz aktivno uključivanje ključnih aktera iz industrije i Vlade, itd.
G. u vezi sa socioekonomskim faktorima: 1. povećanje razumevanja konteksta na lokalnom nivou, društvenog identiteta farmera, briga i mreža uticaja pruža dodatne alate za učenje o politici i načinima za poboljšanje motivacije i ohrabrenje farmera u smislu primene biosigurnosti, 2. sociološka i demografska istraživanja mogu olakšati ciljanje budućih obuka iz biosigurnostii, 3. treba sprovesti dalja istraživanja o uticaju bihejvioralnih determinanata na specifične mere biosigurnosti farme, itd.
H. vezano za posetioce: 1. svi posetioci treba da praktikuju sprovođenje preventivnih strategija specifičnih za farmu, 2. treba obezbediti adekvatne uslove za održavanje biosigurnosti na farmama, zbog razlika između kategorija posetilaca, ali istovremeno povremeni posetioci treba da preuzmu odgovornost i za izbegavanje širenja bolesti, itd.
I. koji se odnose na kreatore politike: 1. propisi koji se odnose na finansijsku podršku i kazne, 2. organizovanje, finansiranje i sprovođenje strogih smernica za kontrolu bolesti kroz Vladinih intervencija, 3. nacionalni standardi i vodiči za planiranje kreirani su da podrže usvajanje prakse biosigurnosti nudeći farmerima planove i strategije biosigurnosti, 6. kazne i nadoknade mogu garantovati pravilno korišćenje praksi biosigurnosti, 7. programe biosigurnosti koji su specifični za proizvođača, 8. jasan zakonski okvir koji daje spisak obaveza i preporuka u pogledu obezbeđenja biosigurnosti na farmama, 9. delovanje na lokalnom nivou može se postići uključivanjem farmera u kreiranje politike biosigurnosti itd.
J. vezano za druge faktore: 1. dalja istraživanja koja će istražiti razloge neuspeha, ciljani obrazovni programi, 2. identifikovanje razlika u stavovima proizvođača da prilagode programe posebno potrebama pojedinaca, dalje razumevanje motivatora i prepreka iza njih. usvajanje praksi biosigurnosnosti, 3. identifikovanje prednosti, slabosti i ograničenja, 4. dizajniranje, implementacija, obuka i praćenje, 5. saveti specifični za stado za poboljšanja dobijeni alatima za procenu biosigurnosti zasnovanim na riziku i poređenjem sa kolegama, 6 promocija plana biosigurnosti treba da se vrši prema geografskom rasporedu regiona, tipu smeštaja, proizvodnji mleka u stadu i organskom statusu, 6. komunikacija sa farmerima i kreatorima politike nakon istraživanja o efikasnosti mera biosigurnosti je veoma bitna, 7. model procene rizika može da ilustruje efekat različitih mera biosigurnosti na nivou farme, 8. studije o merama biosigurnosti koje se sprovode na nivou farme treba da obuhvate zdravlje ljudi i ekonomske rezultate u budućnosti, 8. treba uraditi dalja istraživanja o efikasnosti mera biosigurnosti i definisati jasne preporuke za farmere, 9. dobijanje informacija o strategijama za poboljšanje biosigurnosti doprinosi dugoročnom unapređenju strategija upravljanja stočarstvom, itd.

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Sat 3: Koje bi bile najadekvatnije strategije za poboljšanje komunikacije oko biološke bezbednosti u ovoj oblasti?

Strategije za poboljšanje komunikacije: 1. štampani leci ili pamfleti, 2. edukativni video zapisi, 3. pisana korespondencija (pisma, e-mailovi), 4. telefonski razgovori, 5. individualni onlajn sastanci, 6. internet, vebinari ili onlajn seminari, 7 posete farmama na licu mesta, grupni sastanci licem u lice, 7. onlajn resursi i veb stranice (na primer, veb stranica Uprave za veterinu Ministarstva poljoprivrede, vodoprivrede i šumarstva), 8. agencija/vlada, 9. štampa/farming press, 10. savetnici za osiguranje poljoprivrednih proizvoda, 11. istraživački radovi/časopisi, 12. ostali farmeri, 13. kursevi obuke, 14. poljoprivredne emisije, 15. demonstracione farme, 16. televizija/mediji, 18. prodavci/predstavnici i konferencije, 19. saveti kompanije za farmske životinje, 20. veterinari ili više specijalizovanih konsultanata, kampanje i alati za testiranje biosigurnosti, 21. teorijska vežba rada sa modelom može biti korisna za bolje razumevanje rizika specifičnih za farmu veterinarskih savetnika, ali i za motivisanje farmera da unaprede biosigurnost farme jer je prilagođena svakom farmeru posebno, 22. radio program, 23 programi za kontrolu bolesti, 24. predstavnici mlečnih kompanija, 25. časopisi, 26. članci i sajtovi, 27. demografski relevantni obrazovni programi, 28. grupe za diskusiju farmera, novine i šema osiguranja/kvaliteta na farmi, 29. izvori informacija o biosigurnosti uključuju prisustvovanje otvorenim danima, aktivnosti praćenja/demonstracije; konsultovanje vladinih izvora informacija i konsultovanje predstavnika istraživačkih i obrazovnih organizacija, 30. razgovori sa savetnicima, konsultantima ili veterinarskim stručnjacima su veoma važni za dobijanje informacija o biosigurnosti farme, 31. mentori i sistemi podrške, 31. članci u časopisima, informacije putem organizacija, brošura i aktivan dijalog sa farmerima se predlažu za unapređenje biosigurnosti farme, 32 izvori informacija su veterinari, ostali farmeri, služba za zdravlje životinja, poljoprivredna komora, mobilna aplikacija i mobilno udruženje farmera, 33. evaluaciju koncepta biosigurnosti može uraditi veterinar, onlajn alat ili institucija itd.

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WORKSHOP:

EDUCATIONAL PARTICIPATORY WORKSHOP WITH STAKEHOLDERS: BIOSECURITY PRACTICES ON RUMINANT FARMS AND HOW TO COMMUNICATE IT TO FARMERS

Slavča Hristov, Branislav Stanković, Milica Rađenović*

*University of Belgrade - Faculty of Agriculture, Belgrade-Zemun, Republic of Serbia

S.H. https://orcid.org/0000-0002-1719-9971, B.S. https://orcid.org/0000-0003-3925-6102

Structure of the workshop
Starting point: 1. do you usually discuss preventive measures (biosecurity) with farmers? related to a specific disease? and 2. are you aware of any biosecurity improvement program in the country, related to some specific disease?

Discussion topics in the workshop:
Topic 1**:** Challenges to implementing biosecurity on farms
Topic 2: What is needed to improve biosecurity in ruminant farms of the region?
Topic 3: Which would be the most adequate strategies to improve communication?

Within the Discussion in connection with the first two topics, lists of issues (questions) to be discussed at the workshop were prepared based on the literature and the author's experience.

The list of issues (questions) was sorted into several groups as: related to veterinarians, related to farmers, related to production types, related to farms and pasture, related to visitors, related to collaboration between stakeholders, related to companies and contractors, related to socioeconomic factors, related to policymakers, and related to other factors.

This grouping of issues (questions) will assist in discussions with veterinarians at the workshop.

LISTS OF ISSUES (QUESTIONS) TO BE DISCUSSED AT THE WORKSHOP

Topic 1: Challenges to implement biosecurity on farms:

A. related to veterinarians: 1. Lack of relevant education for veterinarians, veterinarian's perception level of biosecurity can vary, and many of them had no adequate training after graduation, 2. multiple farm visits by veterinarian (vehicle, equipment, practice personnel), 3. many veterinarians did not set time aside to speak about biosecurity plans to producers, 4. veterinarians are not asked questions regarding farm biosecurity, therefore veterinarians assume protocols are being carried out or they often feel that they are not qualified to give advice, 5. lack of clarity of biosecurity information, 6. insufficient evidence for the effectiveness of biosecurity practices, producers and other herd health specialists, 7. a lack of correct information and quantitative data that truly links biosecurity improvement and animal health and production, 8. unclear information about compulsory tests when purchasing animals, 9. no precise definition of biosecurity measure and a lack of understanding of the difference between its' importance and effectiveness, 10. minor disease threat perception depending on the disease history of the region and financial limitations, 11. lack of knowledge about biosecurity effectiveness, but also about improving biosecurity regarding potential disease entry points, etc.
B. related to farmers: 1. lack of understanding about ways of disease transmission, 2. Inadequate knowledge regarding infection routes, lack of education to change attitudes, 3. lack of training: no formal training is provided to new staff on farms, 4. lack of financial resources, 5. psychological barriers and other confounding factors, 6. cost (in time and money), 7. farmers' ability and willingness to invest in biosecurity measures (the major constraint seen by veterinarians), 8. social network or community structure of producers, attitudes and motivators for implementing disease preventive measures, 9. emotional state and

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perceptions of farmers, 10. additional workforce, expected required investment in labour, time and capital, more administration (registration of visitors), rules and inspections, 11. unclear information about compulsory tests when purchasing animals, the cost of the purchase protocol and ignorance, 12. farmers are unaware of the cost and loss of income because of animal diseases, 13. different perception of the risk, 14. the perception of the effectiveness and lack of understanding of biosecurity practices, 15. farmers have little faith in the efficacy of biosecurity measures on farms in the absence of actions of others, such as the government securing borders to prevent illegal risk imports, 16. lack of facilities on farms, lack of farmer cooperation, 17. Farmers' perception of the measures' benefits and health responsibility, as well as low-risk aversion, 18. psycho-socio-demographic factors influence farmers' behaviour in terms of biosecurity implementation, 19. uselessness as perceived by farmers, due to the absence of problems, 20. farmers often do not implement their control measures and rely on visitors' and event organizers' professionalism, 21. Individuals' preparedness for change, and beliefs such as that diseases are inevitable influence the level of farm biosecurity, 22. biosecurity interventions meant for smallholders are not a "one size fits all", therefore pre-defined interventions often do not work out as planned, 23. lack of knowledge about biosecurity effectiveness, but also about improving biosecurity regarding potential disease entry points represents a challenge, etc.

C. related to production types: 1. mixed species rearing can present a challenge for animal farm biosecurity, 2. open rearing system, herd size, 3. a high degree of commingling animals from different farms of origin, 4. herd milk production as a biosecurity risk, 5. Organic status as a biosecurity risk, 6. increased herd size without adaptation of facilities for housing cattle, 7. hobby or small-scale enterprises have low levels of biosecurity on farms, 9. different biosecurity measures are favoured on big and small farms, and they are less implemented on smaller farms, 10. the lowest biosecurity levels are associated with mixed farms, 10. the 'closed herd' concept is misunderstood by farmers, etc.

D. related to farms and pasture: 1. the proximity of farms, 2. geographical region, 3. type of housing, 4. lack of space to maintain a physically separated area, 5. haring equipment, 6. farm infrastructures, available space and herd type influence compartmentation, 7. waste of space and the additional working time needed for the maintenance of an ungrazed corridor, 8. ot being able to prevent contact with wildlife, 9. traditional system of land rental and the high prevalence of farms with associated outlying farms have a negative influence on-farm biosecurity, etc.

E. related to visitors: 1. high number of visitors, 2. high number of visits, 3. different visitors had different conditions for maintaining biosecurity on farms, 4. many visitors reported no access to hand-washing facilities, etc.

F. related to collaboration between stakeholders: 1. farmers have little faith in the efficacy of biosecurity measures on farms in the absence of actions of others, such as the government securing borders to prevent illegal risk imports, 2. farmers, veterinarians and auxiliary industries lack trust in each others' implementation of biosecurity, 3. relationship between farmers and veterinarians, social dynamics, available time and space, farm workers that lack proper training, and mistrust towards the public administration, 4. unharmonised messages and recommendations, negative perception of control authorities, lack of control at other farms/professional visitors/wildlife, no consolidation of legal requirements, a contradiction in measures between sectors, more constraints, increased production costs, and non-competitive prices, etc.

G. related to companies and contractors: 1. farm-level contacts with companies and contractors who do not perform biosecurity prevention practices, 2. the veal companies a lack of information about farm management improvement and implementation, 3. difficulties in communication between farmers, their employees, and visitors, because of problems such as different native languages and dialects, 4. the role of milk buyers and veterinary surgeons is important in the uptake of all types of biosecurity measures, etc.

H. related to socioeconomic factors: 1. uneven on-farm biosecurity practices are transferred differently between social groups, geographical scales and agricultural commodity chains, 2. rural poverty, 3. many rural veterinarians reported biosecurity infrastructures, such as calving areas or isolation stalls, being rarely available on farms, etc.

I. related to policymakers: 1. lack of laws and regulations, 2. waiting for guidelines to follow, 4. excess of legislation, bureaucracy, and a potential loss of farmer's autonomy, 5. inadequate border control, 6.

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ineffective policies and regulation, 7. lack of interest for farm biosecurity implementation is linked to collective attribution, 8. there are strong negative attitudes toward certain sources of information, etc.
J. related to other factors: 1. a long distance from veterinarian services can represent an issue, 2. there is a lack of robust evidence that is needed for recommendations on types of biosecurity interventions for reducing the transmission of bacterial infections from livestock to humans, that are the most effective for improving human health outcomes, 3. unforeseen obstacles, etc.

Topic 2: What is needed to improve biosecurity in ruminant farms of the region?

A. related to veterinarians: 1. providing accessible and practical information on biosecurity, 2. information about simple biosecurity measures and persistent support and a better understanding of costs and loss of income that are caused by diseases, 3. changing the perception of effectiveness through the involvement of veterinarians in discussions about on-farm biosecurity can lead to the adoption of biosecurity practices, 4. improve formal health planning is the solution to biosecurity promotion, 5. improve veterinarians communication skills in order to influence farmers, 6. improve discussions between veterinarian and farmers, 7. address the actual infectious risks, feasibility, and cost-efficiency when recommending biosecurity measures, 8. it is important to emphasise the actual evidence-based benefits, 9. different mental constructs should be addressed by communication strategies, 10. addressing priority issues is a key strategy for adopting biosecurity measures, 11. it is necessary to identify the most important diseases that affect animal farms so that specific measures related to public health purposes can be introduced, 12. biosecurity must be more included in veterinary training programs because veterinarians should play a more active role in terms of guidance and improvements of biosecurity implementation at farm level, 13. rural veterinarians need a proper guidance from the authorities, a proper training on biosecurity and communication, as well as an enabling environment, 14. knowledge of veterinary epidemiology and understanding of disease transmission and spread, risk factors and methods to prevent disease are important for the development of control programs for diseases and implementation of biosecurity programs at a farm, regional and national level, 15. there is a need to create farm-specific preventive strategies in collaboration with herd veterinarians, 16. contingency planning of emerging diseases or combating endemic diseases can be facilitated using the information about which biosecurity measures are in use, 17. training should be provided with a bigger involvement of veterinarians and should be tailored to defined groups and specific needs, with the use of new technologies and recording systems, 18. preferably training should be in a face-to-face format, 19. tailoring information dissemination strategies, enhancing access to veterinary services, and prioritizing education in key areas, 20. a closer alignment of communication strategies with the preferences of the target audience can maximize biosecurity information uptake, 20. active dialogue with farmers is recommended for better biosecurity implementation, 21. it is important to provide development assistance, engage smallholders who are willing to improve systems, allow sceptical smallholders to observe and participate at their convenience timing, and link biosecurity with gains such as income, and time-saving, etc.
B. related to farmers: 1. Increase awareness - better communication, 2. more training, 3. more resources to improve infrastructure, 4. training in developing biosecurity plans , 5. education of farmers should be enhanced through training which would highlight the importance of biosecurity practices, 6. improve acceptance and enforcement of biosecurity practices by improving farmers' education, 7. training programs to change producers' attitudes and perceptions concerning poor biosecurity practices, 8. limiting the farms of purchase of animals, 9. there is a need in improvement in farmer knowledge of large ruminant health and production, 10. applied field research, “on the job” training and “formal” training programmes provide necessary information to farmers, 11. documenting the implementation of on-farm biosecurity can be beneficial for countries facing farm expansion, 12. better access for farmers to biosecurity information and advice sources should be ensured, 13. it is important to provide development assistance, engage smallholders who are willing to improve systems, allow sceptical smallholders to observe and participate at own convinient timing, and to link biosecurity with gains such as income, and time saving, etc.
C. related to production types: 1. biosecurity measures based on individual characteristics of the farm could help to improve farm biosecurity, 2. smaller farms should have higher levels of biosecurity, therefore counselling regarding potential risks is recommended, etc.

D.**related to farms:** 1. identify what is feasible at farms, 2. village-level biosecurity programmes should be introduced, 3. facilities that are already present on farms should be used more often, 4. the level of biosecurity of a farm is a very important indicator of potential improvement, therefore it should be assessed, 6. better attention should be paid to prevention of direct contact with cattle from other herds, etc.

E. related to collaboration between stakeholders: 1. collaborative action and taking responsibility amongst all operators within the industry should be promoted by the public sector, 2. improved decision support tools which will make benefits of improved farm biosecurity more apparent, 3. identifying the communication gaps between research and industry and adress it in form of trainings for veterinarians, 4. establishing good communication channels between farmers, veterinarians and other herd health advisors is necessary, 5. promoting education about infectious diseases and disease prevention measures among farmers, 6. training should be participatory and should involve communities, but also encourage participants to overcome practical and cultural obstacles in order to enhance the farm biosecurity level, 8. new approach is required to forge collective behavior in terms of biosecurity implementation, therefore neutral communication should be encouraged, etc.

F. related to companies and contractors: 1. distribution of cleaning and disinfection products by the companies, 2. the different stakeholders should take action regarding biosecurity measures, 3. the owners, managers, and workers in livestock enterprises, industry bodies, and rural and urban communities should be involved in training regarding biosecurity, 4. a multidisciplinary approach should be used to address the improvement of on-farm biosecurity with the active involvement of key industry and government stakeholders, etc.

G. related to socioeconomic factors: 1. increasing understanding of local-level context, farmers' social identities, practices of care, and networks of influence gives additional policy learning tools and ways to improve motivation and encourage farmers in terms of implementing biosecurity, 2. sociological and demographic research can facilitate the targeting of the future trainings in biosecurity, 3. further research about the effect of behavioral determinats on specific farm biosecurity measures should be carried out, etc.

H. related to visitors: 1. the implementation of farm-specific preventive strategies should be practiced by all visitors, 2. adequate conditions regarding maintaining biosecurity on farms need to be provided, because of the differences between the visitor categories, but at the same time visitors should take responsibility for avoiding disease spread, too, etc.

I. related to policymakers: 1. regulations regarding financial support and penalties, 2. organizing, funding, and enforcing strict disease control guidelines through government intervention, 3. national standards and producer planning guides are created to support the adoption of biosecurity practices by offering biosecurity plans and strategies, 6. penalties and compensations may guarantee the proper use of biosecurity practices, 7. biosecurity programs that are producer-specific, 8. a clear legal framework providing the list of obligations and recommendations in terms of biosecurity at cattle farms should be provided, 9. action at the local level can be achieved by farmer involvement in the design of biosecurity policy, etc.

J. related to other factors: 1. further research that will explore the reasons for the lack of uptake, targeted education programs, 2. identifying differences in producers' attitudes to tailor programs specifically to individuals' needs, further understanding of motivators and barriers behind the uptake of biosecurity practices, 3. identifying strengths, weaknesses, and constraints, 4. designing, implementing, training, and monitoring, 5. herd-specific advice for improvements obtained by risk-based weighted biosecurity evaluation tools and comparison with peers, 6. the promotion of the biosecurity plan should be done according to geographical region, type of housing, herd milk production, and organic status, 6. communication with farmers and policymakers after research on the effectiveness of biosecurity measures, 7. the risk assessment model can illustrate the effect of different biosecurity measures on farm level, 8. studies on biosecurity measures implemented at the farm levels should include human health and economic outcomes in the future, 8. further research should be done about the effectiveness of biosecurity measures and clear recommendations for farmers, 9. gaining information about strategies for biosecurity improvement contributes to long-term strategies for livestock-improved management, etc.

Topic 3: Which would be the most adequate strategies to improve communication around biosecurity in the area?

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Strategies to improve communication: 1. printed leaflets or pamphlets, 2. educational videos, 3. written correspondence (letters, e-mails), 4. telephone conversations, 5. individual online meetings, 6. internet, webinars or online seminars, 7. on-site farm visits, face-to-face group meetings, 7. nline resources and websites (for example, the website of the Veterinary Administration of the Ministry of Agriculture, Water Management and Forestry), 8. agency/government, 9. press/farming press, 10. farm assurance advisors, 11. research papers/journals, 12. other farmers, 13. training courses, 14. agricultural shows, 15. demonstration farms, 16. television/media, 18. salesmen/reps and conferences, 19. farm animal company advice, 20. herd veterinarian or more specialized consultants, campaign, and biosecurity testing tools, 21. the theoretic exercise of working with the model can be useful for a better understanding of farm-specific risks by veterinary advisors, but also for motivating farmers to improve farm biosecurity because it is tailored to each farmer specifically, 22. radio programme, 23 disease control programs, 24. dairy company representatives, 25. magazines, 26. articles and websites, 27. demographically relevant education programmes, 28. the farmer discussion group, the newspaper, and the farm assurance/quality scheme, 29. biosecurity information sources include attending open days, monitoring/demonstration activities; consulting Government information sources, and consulting representatives of research and educational organisations. 30. discussions with advisors, consultants or veterinary surgeons are very important for gaining information about farm biosecurity, 31. mentors and support systems, 31. journal articles, information via organizations, brochures, and active dialogue with farmers are suggested for the improvement of on-farm biosecurity, 32 sources of information are veterinarians, other farmers, the animal health service, the chamber of agriculture, the mobile app, and the mobile stable association, 33. evaluation of the biosecurity concept can be done by a veterinarian, an online tool or an institution, etc.

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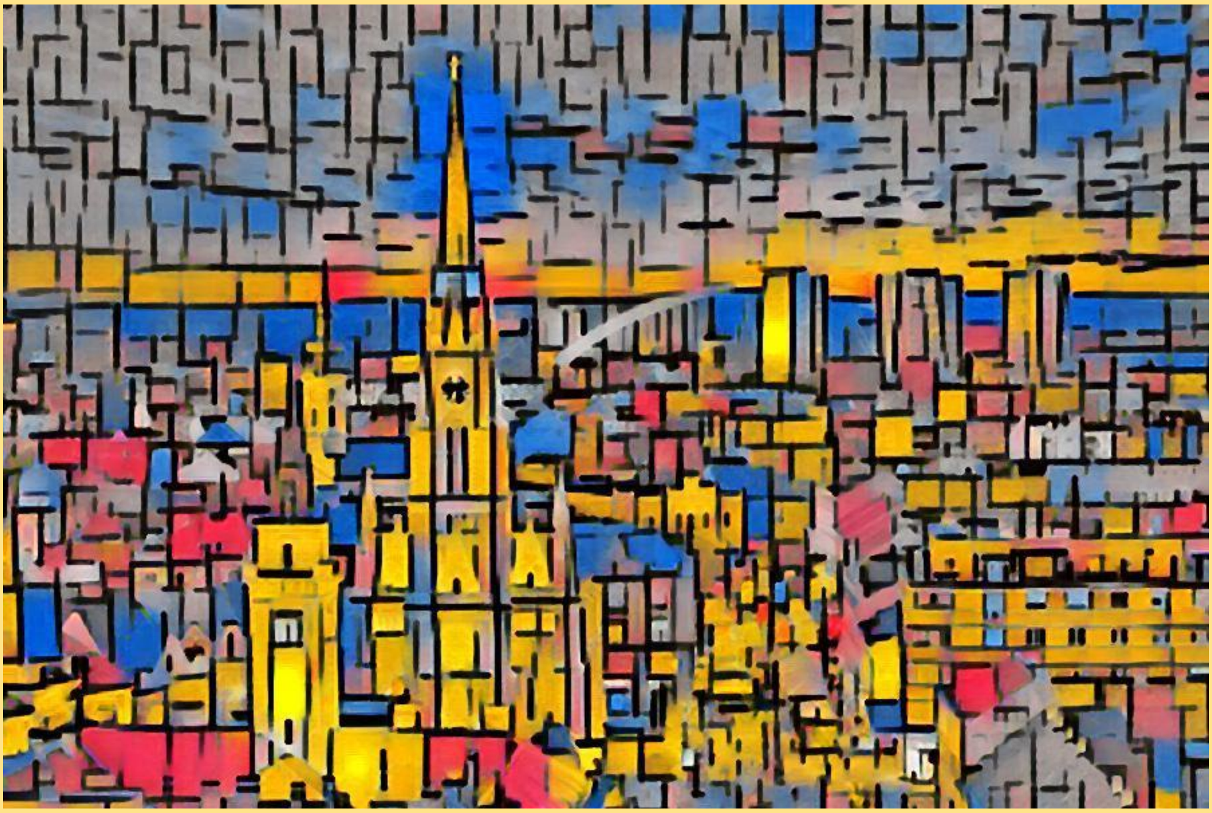

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