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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

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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

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.

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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