26TH INTERNATIONAL CONGRESS OF THE MEDITERRANEAN FEDERATION FOR HEALTH AND PRODUCTION OF RUMINANTS FeMeSPRum, Novi Sad (Serbia), 20th – 23rd June, 2024
26. MEĐUNARODNI KONGRES MEDITERANSKE FEDERACIJE ZA ZDRAVLJE I PRODUKCIJU PREŽIVARA FeMeSPRum, Novi Sad (Srbija), 20. – 23.jun 2024.godine
ISBN 978-86-7520-611-8

DOI 10.5937/FeMeSPRumNS24016J Article
UDC 546.56 Originalni rad

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

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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

ISBN 978-86-7520-611-8

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

ISBN 978-86-7520-611-8

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.

Acknowledgments

The authors gratefully acknowledge financial support from the Slovenian Research Agency and the Ministry of Agriculture, Forestry and Food within the project Strategic Approaches to Improve Health Status and Fertility of Small Ruminants, core funding research no. (V4-2005).

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

ISBN 978-86-7520-611-8

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

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