Evaluation of Hematological Parameters and Trace Elements in the Blood of Sheep

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Abstract
The ewes were kept at three farms. Blood samples were divided according to factors of Altitude (550 m, 800 m, 950 m), Season (spring, autumn), Breed (Charolais, Merinolandschaf, Sumavian sheep), and System (ecological, non-ecological). The lowest haemoglobin concentration was detected at altitude 550 m (66.95 $g.L^{-1}$) and the highest at the altitude of 950 m (115.54 $g.L^{-1}$) ($P<0.001$). The highest content of haemoglobin was recorded in Sumavian sheep (115.54 $g.L^{-1}$) ($P<0.001$) and in the ecological system (115.65 vs. 85.80 $g.L^{-1}$, $P<0.001$; 0.39 vs. 0.36 $L.L^{-1}$, $P<0.05$). The highest number of leucocytes was at the altitude of 950 m (9.44 G. L^{-1}) ($P<0.001$) and in the spring season (8.97 G. L^{-1} vs. 6.47 G. L^{-1}), and in the ecological system (9.57 G. L^{-1} vs. 7.13 G. L^{-1}) ($P<0.001$). The highest percentages of eosinophiles were found at the altitude of 550 m (9.26) ($P<0.05$) and in the Merinolandschaf breed (9.89) ($P<0.01$). The highest copper concentration was found at the altitude 550 m and the lowest at the altitude 800 m (17.07 $\mu mol.l^{-1}$ vs. 13.58 $\mu mol.l^{-1}$) ($P<0.001$). Zinc levels at altitudes 800 m and 950 m were higher than at altitude 550 m (17.53 $\mu mol.l^{-1}$, 17.93 $\mu mol.l^{-1}$ vs. 14.77 $\mu mol.l^{-1}$) ($P<0.001$). Zinc concentration was lower in the spring then autumn (15.08 $\mu mol.l^{-1}$ vs. 17.00 $\mu mol.l^{-1}$) ($P<0.01$). The ecological system contained lower levels of copper (14.49 $\mu mol.l^{-1}$ vs. 16.14 $\mu mol.l^{-1}$) ($P<0.01$) and higher levels of zinc (17.81 $\mu mol.l^{-1}$ vs. 15.22 $\mu mol.l^{-1}$) ($P<0.01$).

Keywords: copper, hematology,sheep, zinc.

1. Introduction

Normal physiological values of different blood parameters for animals are influenced by a number of factors such as age, sex, breed, season, altitude, climatic conditions, nutrition and life habits of the species [1] [2] [3]. Assessment of trace element status identifies whether current mineral supplementation of livestock feed is adequate and whether improved productivity is likely to occur with changes in supplementation. Some metals are essential for life, others have unknown biological function, either favorable or toxic, and some others have the potential to produce disease [4].
Those causing toxicity are the ones, which accumulate in the body through food chain, water and air [5].The efficiency of sheep is obviously dependent on the health and the well-being of the ewe.
Mineral deficiencies, and in some cases imbalances, cause metabolic disturbances and can produce specific deficiency diseases [6] [7].
Copper has a multiple function, as iron absorbtion, haemopoiesis, and various enzyme activities and in the oxidation-reduction process [8]. Phagocytic ability of neutrophils was increased when copper was administered to copper deficient goat kids [9]. Copper administration can cause significant increase in haemoglobin and serum copper levels. The dietary copper deficiency has proinflammatory effects on neutrophils and the microvascular endothelium that promote neutrophil-endothelial interactions [10].
Zinc plays an essential role in a number of catalytic, structural and regulatory functions, RNA, DNA, ribosome stabilization. Zinc

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deficiency results in disorders of testicular development and spermatogenic failure [5]. Slavik [11] demonstrated insufficient concentrations of zinc and copper in forage and deficiencies in beef cattle from the Sumava region.

2. Materials and methods

The objective of the study was to determine hematological measures and copper and zinc plasma concentrations of sheep in selected herds kept in a mountainous region. We tested the hypotheses that observed parameters are impacted of altitude, season, breed, management system, and herd in blood plasma of sheep.

The adult female sheep (2-4 years) were kept in three farms. Blood samples were divided according to factors Altitude (550 m above sea level; 800 m above sea level; 950 m above sea level), Breed (Charolais, Merinolandschaf, Sumavian sheep), System (ecological, non-ecological). Blood samples were collected during spring and autumn in two years, therefore, they were divided according to two periods: spring and autumn).

The observations were located at the South-west region of Czech Republic. The animals were fed on pasture grass and they had ad libitum access to water and free-choice mineral supplement. Mineral treatments were provided at a single location in each pasture in free-choice mineral feeders. Feed intake was monitored daily in each observation for 3 days.

Sheep were managed in experimental farm 1 located at the 550 m above sea level with non-ecological system. Basic herd of 70 ewes Charolais breed is housed in barn during winter. During the grazing season, the animals are kept on pastures with access to a cowshed. Size of herd in the Farm 2 was 90 Merino-landschaf ewes, non-ecological system. The animals grazed outdoors on pasture at elevation 800 m. During the winter, the sheep were kept in the wood barn with access to an outdoor pen and fed locally produced hay.

Farm 3 located in hilly mountain region Sumava is divided on two herds kept at the 950 m above sea level with ecological system. Ewes Sumavian breed were grazed outdoors on pasture throughout the year in surrounding paddocks and in winter, they are fed locally produced hay in the shelter.

Blood samples were collected in the third day of observation by jugular venipuncture into heparinized tubes. Leukocytes count was determined using a dilution method and Bürker chamber, the content of haemoglobin was estimated photometrically at 540 nm by using a spectrometer UV/VIS Unicam 5625. The haematocrit value was determined by capillary microhaematocrit method according to Janetzki. The phagocytic activity of sheep was determined via phagocytosis percentage (% phagocytosis). The phagocytosis percentage was determined as a ratio of phagocytized neutrophiles leukocytes and the total leukocyte counts, multiplied by 100.

The concentration of copper and zinc in blood plasma, and in dry matter of a diet was analysed by flame atomic absorption method using an AA Spectrometer Unicam 969.

The data were analysed with a statistical package STATISTIX, Version 8.0 (Anonymous, 2001).

3. Results and discussion

The lowest haemoglobin concentration was at the altitude 550 m (66.95 ± 2.35 $g.L^{-1}$) and the highest at the altitude 950 m (115.54 ± 6.59 $g.L^{-1}$) ($P<0.001$). Differences among individual altitudes were very highly significant. The haematocrit level was higher at altitude of 3 (950 m above sea) than at altitude 550 m (0.39 ± 0.01 $l.l^{-1}$ vs. 0.36 ± 0.01 $L.L^{-1}$; $P<0.05$). The factor of Season significantly differed only in the case of haematocrit (0.36 ± 0.01 $L.L^{-1}$vs. 0.39 ± 0.01 $L.L^{-1}$ ($P<0.05$). The highest content of haemoglobin was recorded in the Breed 3 (115.54 ± 6.64 $g.L^{-1}$); differences among breeds were significant ($P<0.001$). The significant differences were also between systems in both parametres, higher content of haemoglobin and haematocrit were found in the ecological system (115.65 ± 6.71 vs. 85.80 ± 3.88 $g.L^{-1}$; 0.39 ± 0.01 vs. 0.36 ± 0.01) ($P<0.05$).

The highest count of leucocytes was recorded at the Altitude 950 m (9.44 ± 0.59 $G.L^{-1}$) ($P<0.001$). The higher counts of leucocytes were contained in the spring season (8.97 ± 0.33 G. L^{-1} vs. 6.47 ± 0.33 G. L^{-1}) and in the ecological system (9.57 ± 0.48 G. L^{-1} vs. 7.13 ± 0.28 G. L^{-1}) ($P<0.001$).

The lowest percentage of eosinophiles was found at the altitude 800 m (5.50 ± 0.95 %) and the highest at the altitude 550 m (9.26 ± 0.67) ($P<0.05$). We found the lowest percentage of eosinophiles in the Charolais breed (8.55 ± 0.72

%) and the highest in the Merinolandschaf breed (9.89 ± 0.88) (P<0.01).
Activities of phagocytose significantly differed in the factor of Altitude, the highest value was recorded at the altitude 950 m (95.26 ± 3.11 %) and the lowest value at the altitude 550 m (85.12 ± 1.30 %) (P<0.001). There were higher phagocytose activities in the autumn season (90.90 ± 1.28 %) (P<0.01) and in the ecological system (95.07 ± 1.97 %) (P<0.001).
The highest haemoglobin concentration was recorded in the altitude of 950 m, in Sumavian sheep and also in the ecological system. These findings are closely correlated. Breed differences for these parameters were also reported earlier [12], however, we did not find a comparison among breeds Charolais, Merinolandschaf, and Sumavian sheep. The effect of altitude on erythrocytic values has been studied by many investigators and it is now a well established fact that reduced oxygen tension, in highland regions, leads to an increased production and release of erythropoietin, thereby, stimulating erythropoiesis as a coping or adaptive mechanism to low oxygen level in such an environment [13]. Therefore, the higher haemoglobin and haematocrit values exhibited in observed altitude of 950 m above sea level in this study could provide evidence of the adaptation of these sheep to lower amount of atmospheric oxygen.
The highest number of leucocytes was again at the altitude of 950 m. The higher counts of leucocytes were recorded in the spring season and in the ecological system. Mechanisms of action have not been identified. However, possession of a haemoglobin with high oxygen affinity helps to adapt animals to high altitudes [14]. We can only suppose that the higher leukocyte count was caused by adaptation.
Eosinophil concentrations were found highest in the blood of ewes at the altitude of 550 m and in the Merinolandschaf breed, but values at the other altitudes or breeds were not low enough to be deemed typical symptoms of stress [15]. The highest value of phagocytose activity was recorded in the altitude of 950 m and the lowest value in the altitude 550 m. This might be attributed to a better adaptation of the organism in a mountainous environment. There were higher phagocytose activities in the autumn season and in the ecological system.
The highest copper concentration was found at the altitude 550 m and the lowest at the altitude 800 m (17.07 ± 0.25 μmol.l^{-1} vs. 13.58 ± 0.35 μmol.l^{-1}). Zinc levels were higher at altitudes of 3 (950 m above sea) and 2 (800 m above sea) than at altitude 550 m above sea level (17.93 ± 0.97 μmol.l^{-1}, 17.53 ± 0.64 μmol.l^{-1} vs. 14.77 ± 0.46 μmol.l^{-1}). Differences among altitudes were very highly significant in both parameters. There was recorded zinc concentration difference between spring and fall in the factor Season (15.08 ± 0.49 μmol.l^{-1} vs. 17.00 ± 0.49 μmol.l^{-1}) (P<0.01).
Factor Breed was differed in the both microminerals (P<0.001). The highest value of copper was measured in the Charolais sheep (17.64 ± 0.30 μmol.l^{-1}) (P<0.001) and the lowest concentration in the Merinolandschaf sheep (14.17 ± 0.36 μmol.l^{-1}); the highest content of zinc was in the Sumavian breed (17.98 ± 0.91 and the lowest in the Charolais sheep (14.68 ± 0.53 μmol.l^{-1}) (P<0.01). Factor System differed in the case of copper (14.49 ± 0.46 μmol.l^{-1} vs. 16.14 ± 0.26 μmol.l^{-1}) (P<0.01) and also in the zinc (17.81 ± 0.71 μmol.l^{-1} vs. 15.22 ± 0.40 μmol.l^{-1}) (P<0.01).
The highest copper concentration was found at the altitude of 550 m above sea level. Copper content in the sheep under study was not marginal, we did not find the copper deficiency farms with exception of October 2005 observation (1.82 mg.kg^{-1} in dry matter), just exactly at the same altitude of 550 m. Higher copper intake could be causing by the extraordinary consumption of mineral licks. The mineral supplementation of diets is almost ubiquitous in lactating ewes (Sumavian sheep), but is much less routine in non lactating herds. Moreover, the trace mineral status of animals depends not only on dietary allowance, but also on the efficiency of digestion and storage, which both can be affected by interactions with other food constituents.
The highest value of copper was measured in the Charolais sheep and the lowest concentration in the Merinolandschaf sheep, the highest content of zinc was in the Sumavian breed and the lowest in the Charolais sheep. In the ecological system were lower levels of copper and higher levels of zinc.

There were higher copper concentration in non-ecological system and lower zinc concentration. However, copper levels in sheep were generally high in this place (Charolais). Even though we did not record toxic values and the concentrations measured for plasma copper were in the range used by veterinary laboratories, animals in some areas could be potentially exposed to environmental contaminants [16] [17]. Zinc levels in blood plasma were similar to those reported in most other studies.

In comparison to copper plasma concentration, zinc levels were on the opposite end of the spectrum. The lowest zinc content in blood plasma was at the altitude 550 m, although dry matter feed contained the highest amount of zinc (61.7 $mg.kg^{-1}$). The content of microelements copper and zinc per 1 kg of dry matter of fodders was similar to the recommendation from Sommer [18] standards.

The zinc concentration was lower in spring then autumn. The toxic effects of risk elements on sheep may become evident in health disturbances and decreased performance and reproductive indices. However, we did not record any health problems in the observed animals.

Conclusions

Results indicated that the content of minerals in different plant fodders given to ewes could be diversified. Therefore, their imbalance in the diet could lead to differences in observed parametres in blood plasma. We can conclude that hematological markers and trace mineral contents may be impacted of altitude, season, breed, and management system in grazed sheep.

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