Biologia **66**/1: 181—186, 2011
Section Zoology
DOI: 10.2478/s11756-010-0150-3

Hematology and blood microelements of sheep in south Bohemia

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Abstract: The efficiency of sheep is dependent on their health and well-being. The blood markers can be critical for improving of the physiological, nutritional and pathological status of sheep organism. The aim of this study was to test the hypotheses that the red and white blood cells and copper (Cu) and zinc (Zn) plasma contents are impacted by altitude and season. The ewes were kept at three farms. Blood samples were divided according to factors of altitude (550 m, 800 m, 950 m above sea level), season (spring, fall) and year. The lowest haemoglobin concentration and value of haematocrit were detected at the altitude of 550 m (66.95 g L^{-1}, 0.36 L L^{-1}) and the highest at the altitude of 950 m (117.96 g L^{-1}, 0.39 L L^{-1}) ($P < 0.001$). Spring values of haemoglobin and haematocrit were lower than fall values. The highest count of leucocytes was recorded at the altitude 950 m (9.57 G L^{-1}), higher counts were contained in spring ($P < 0.001$). The lowest percentage of eosinophiles was found at the altitude of 800 m (5.81%) and the highest at the altitude of 550 m (9.26%) ($P < 0.01$). Phagocytose activities were the highest at the altitude of 950 m (95.07%) and the lowest at the altitude of 550 m (85.04%) ($P < 0.001$). Phagocytose activities were higher in fall than in spring. The highest Cu concentration was found at the altitude of 550 m and the lowest at the altitude of 800 m (17.04 µmol L^{-1} vs. 14.37 µmol L^{-1}). Zn levels were higher at altitudes of 950 m and 800 m than at the altitude of 550 m (17.81 µmol L^{-1}, 17.00 µmol L^{-1} vs. 14.77 µmol L^{-1}). We concluded that hematological markers and trace mineral content in grazed sheep may be impacted by altitude and season.

Key words: sheep; hematology; copper; zinc

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 (Jelinek et al. 1996; Frelich et al. 2006; Tripathi et al. 2008). Assessment of the trace element status determines whether the current mineral supplementation of livestock feed is adequate and whether an improved productivity is likely to occur with changes in supplementation (Solaiman et al. 2006a). Some metals are essential for life, others have unknown biological functions, either favorable or toxic, while the others have a potential to cause a disease.

The efficiency of sheep is obviously dependent on the health and the well-being of the ewe. More information is needed on their micromineral requirements in relation to nutrition, toxicology, and physiological status. Appropriate trace mineral supplementation is essential for maintaining an optimum level of growth, health and performance of animals (Orden et al. 2000; Kellogg et al. 2004; Solaiman et al. 2006b). Zinc (Zn) and Copper (Cu) have been shown to play a role in the immune response of cattle (Leeson 2009). It is an established fact that nutritional status contributes to an overall immune response of individuals. Mineral deficiencies cause metabolic disturbances and can cause specific deficiency diseases (Rajčáková et al. 2003; Čermák et al. 2006). Cu and Zn deficit present as early as during pregnancy of the mother may have a negative impact on the development of the foetus and its postnatal health (Kellogg et al. 2003; Massanyi et al. 2003b).

Cu is an essential component of several enzymes that are required to maintain host homeostasis and may perform several functions in the immune system measured by phagocytosis or others, whose direct mechanism of action is not clear (Leeson 2009). Microcytic hypochromic anemia is one of the outcomes of Cu deficiency (Swenson & Reece 2004). Cu has multiple functions, such as iron absorption, haemopoiesis, and various enzyme activities and in the oxidation-reduction process. The phagocytic ability of neutrophils was increased when Cu was administered to Cu deficient goat kids. Dietary copper deficiency increases the accumulation of circulating neutrophils in the lung microcirculation (Solaiman et al. 2007).

It is well known that Cu influences erythropoiesis and also biosynthesis of haem and haemoglobin. Cu administration can cause a significant increase in haemoglobin and serum Cu levels (Sahin et al. 2009).

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Table 1. Consumption feeds (kg) and mineral content in dry matter (mg kg^{-1}).

Farm	Observation	Permanent pasture (kg)	Grass hay (kg)	Oats grain (kg)	Copper mg kg^{-1}	Zinc mg kg^{-1}
1	May 2004	1.82	–		7.21	57.12
	October 2004	1.18	0.93		4.33	51.04
	May 2005	2.29	-		9.14	64.52
	October 2005	1.33	0.64		1.82	74.10
2	May 2004	1.75	1.90	0.20	4.38	62.00
	October 2004	0.97	1.28	–	3.26	59.60
	May 2005	1.52	1.76	0.18	5.37	35.52
	October 2005	1.28	1.43	–	4.04	38.68
3	May 2004	2.17	–		5.97	29.06
	October 2004	1.03	0.82		4.30	66.90
	May 2005	1.50	0.62		9.53	32.10
	October 2005	1.03	1.10		8.65	63.57

Results of Lominadze et al. (2004) support the theory that dietary Cu deficiency has proinflammatory effects on neutrophils and the microvascular endothelium that promote neutrophil-endothelial interactions.

Zn plays an essential role in a number of catalytic, structural and regulatory functions, as well as in RNA, DNA, and ribosome stabilization (Massanyi et al. 2001). Šrejberová et al. (2008) found significant correlations between hematological parameters and mineral elements in beef cattle. The study of Slavik et al. (2006) demonstrated insufficient concentrations of Zn and Cu in forage and deficiencies in beef cattle from the mountain regions.

The aim of the study was to determine hematological measures and Cu and Zn plasma concentrations of sheep in selected herds kept in a mountainous region (the Šumava Mts). We tested the hypothesis that observed parameters in the blood plasma of sheep are influenced by altitude and season.

Methods

Adult female sheep (2–4 years) were kept in three farms. Blood samples were separated according to altitude (550 m, 800 m, and 950 m a.s.l.). Blood samples were collected during spring and fall over the course of two years and were separated also according to two seasons: spring and fall.

The observations were conducted in the Southwestern region of the Czech Republic. Grassland is based on the granodiorite geological footwall of the weinsberg type in the internal zone of the South Bohemian Massif. The animals were fed on pasture grass, with an ad libitum access to water and a 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 three days. Feed samples were dried and ground in a mill to pass through a 1 mm mesh screen. In summer, all animals grazed only without additional feeding. The amount and source of microelements in daily rations for sheep are presented in Table 1. The sheep were in good condition during the whole experiment and clinically, including the udder, they were healthy.

Farm 1. Sheep were managed at an experimental farm located at 550 m a.s.l. with a non-ecological system. The herd of 70 Charolais breed ewes was housed in a rock wall barn with clay floor and straw bedding during winter. During the grazing season, the animals were kept on pastures with a clay fluvial soil (683 mm of precipitation per year, 7.2 °C mean temperature per year) with an access to a cowshed. Animals were rotated among pastures approximately every 28 days in order to minimize pasture effects and were fed on hay. During winter feeding, the sheep were given meadow hay and oats.

Farm 2. The herd comprised of 90 Merino-landschaf ewes and were managed in a non-ecological system. The sampled animals rotationally grazed outdoors on pasture at the elevation of 800 m a.s.l. throughout the year. Animals were rotated among pastures approximately every 28 day in order to minimize pasture effects. During the winter, the sheep were kept in a wood barn with access to an outdoor pen and fed locally produced hay. The climate is mild, the average yearly temperature was 6.5 °C, and total rainfall standed at 812.0 mm.

Farm 3. Located in the hilly mountainous region of the Šumava Mts, the farm was divided into two herds kept at 950 m a.s.l. and managed in an ecological system. Ewes of the Šumavian breed were grazed outdoors on pastures throughout the year in surrounding paddocks; in winter they were fed locally produced hay in the shelter. The animals were kept on pastures with light sandy soils (753 mm of precipitation per year, 6.2 °C mean temperature per year). Animals were rotated among pastures approximately every 30 days in order to minimize pasture effects. The farm had been certified for 5 years under the guidelines for organic farming.

General health parameters were obtained on each of the three days of observation. Vital body signs recorded were respiration rate, heart rate and rectal temperature. Blood samples were collected on the third day of the observation by jugular venipuncture into heparinized tubes. The plasma was separated; samples were placed on ice immediately after collection, transported to the laboratory, and stored at – 24 °C until processing.

The haematological parameters were determined as follows: 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 the Janetzki capillary microhaematocrit method. The phagocytic activity of blood cells was determined via phagocytosis percentage (% phagocytosis) using the MSHP kit (microsferic hydrophilic particles for determination of the blood leucocytes phagocytic activity in vitro,

Table 2. Haemoglobin content and haematocrit values in sheep.

Factor	Haemoglobin (g L^{-1})			Haematocrit (L L^{-1})		
	N	$\bar{x} \pm$ SD	Significance	N	$\bar{x} \pm$ SD	Significance
Altitude 1	89	66.95 ± 55.09	***	89	0.36 ± 0.05	***
2	44	82.91 ± 13.20	1 : 2,3***	44	0.38 ± 0.04	1,2 : 3***
3	43	117.96 ± 10.94		43	0.39 ± 0.06	
Season 1	87	91.40 ± 47.35	**	87	0.35 ± 0.05	***
2	89	96.18 ± 49.53		89	0.39 ± 0.04	
Year 1	88	96.16 ± 45.14	*	88	0.36 ± 0.05	*
2	88	91.63 ± 51.17		88	0.39 ± 0.04	

Explanations: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; Altitude: 1 – 550 m, 2 – 800 m, 3 – 950 m; Season: 1 – spring, 2 – fall. NS – non significant. Interactions: Altitude*Season ($P < 0.01$); Altitude*Season ($P < 0.001$); Altitude*Year ($P < 0.001$); Season*Year ($P < 0.05$).

Table 3. Percentage of monocytes and leucocytes counts in sheep.

Factor	Monocytes (%)			Leucocytes (G L^{-1})		
	N	$\bar{x} \pm$ SD	Significance	N	$\bar{x} \pm$ SD	Significance
Altitude 1	89	1.56 ± 1.64	NS	89	6.85 ± 3.08	***
2	44	1.17 ± 1.37		44	7.71 ± 2.16	1 : 3***
3	43	1.57 ± 1.64		43	9.57 ± 4.47	2 : 3**
Season 1	87	1.65 ± 1.62	NS	87	8.97 ± 2.03	***
2	89	1.28 ± 1.52		89	6.47 ± 2.14	
Year 1	88	1.44 ± 1.60	NS	88	7.90 ± 3.47	NS
2	88	1.49 ± 1.56		88	7.59 ± 3.46	

For explanations see Table 2. Interactions: Altitude*Season ($P < 0.01$); Altitude*Season ($P < 0.001$); Altitude*Year ($P < 0.05$).

code RK 031; firm Artim s.r.o., Prague). The phagocytosis percentage was determined as a ratio of phagocytizing neutrophiles and the total leukocyte counts, multiplied by 100.

The concentration of Cu and Zn in blood plasma and in dry matter of a diet was analyzed by the flame atomic absorption method using an AA Spectrometer Unicam 969.

The data were analysed using a General Linear Model ANOVA (three ways with the interactions) via the statistical package STATISTIX, Version 9.0 (Anonymous 2008). The normal distribution of data was evaluated via the Wilk-Shapiro/Rankin Plot procedure. The dependent variables were hematological parameters, copper and zinc concentrations; the independent variables were altitude, season, and year. Significant differences among means were tested via the Bonferroni's test. Significant differences between groups were tested via Comparisons of Mean Ranks. All values are reported as means ± standard deviation of the mean.

Results

The lowest haemoglobin concentration was at the altitude of 550 m (66.95 ± 55.09 g L^{-1}) and the highest at the altitude of 950 m (117.96 ± 10.94 g L^{-1}) ($P < 0.001$). Differences between the values measured at individual altitudes were highly significant (Table 2). The haematocrit level was higher at the altitude of 950 m than at 550 m (0.39 ± 0.06 L L^{-1} vs. 0.36 ± 0.06 L L^{-1}; $P < 0.001$). The effect of season was significant in both markers; spring values of haemoglobin and haematocrit were lower than fall values (91.40 ± 47.35 g L^{-1} vs. 96.18 ± 49.53 g L^{-1}, $P < 0.01$; 0.35 ± 0.05 L L^{-1} vs. 0.39 ± 0.04 L L^{-1}, $P < 0.001$). Interactions in haemoglobin and haematocrit were recorded between Altitude*Season ($P < 0.01$), Altitude*Year ($P < 0.001$), and Altitude*Season ($P < 0.001$) and Season*Year ($P < 0.05$).

No differences between monocyte percentages were found (Table 3). In monocyte counts, we observed interactions only between Altitude*Season ($P < 0.01$) and Altitude*Year ($P < 0.05$). The highest leucocyte count was recorded at the altitude of 950 m (9.57 ± 4.47 G L^{-1}) ($P < 0.001$). Generally, higher counts of leucocytes were observed in spring (8.97 ± 2.03 G L^{-1} vs. 6.47 ± 2.14 G L^{-1}) ($P < 0.001$) (Table 3), thus interactions between Altitude*Season ($P < 0.001$) were found.

The lowest percentage of eosinophiles was found at the altitude of 800 m (5.81 ± 5.10%) and the highest at the altitude of 550 m (9.26 ± 7.62%) ($P < 0.01$). No significant differences were recorded between season and year. Interaction in eosinophile percentages were found between Altitude*Season ($P < 0.01$) and Season*Year ($P < 0.05$) (Table 4). Phagocytose activities significantly differed at various altitudes; the highest value was recorded at 950 m (95.07 ± 7.39%) and the lowest value at 550 m (85.04 ± 13.06%) ($P < 0.001$) (Table 4). Seasonal differences in phagocytic activities were also found; higher phagocytose activities were observed in fall (90.90 ± 9.81%) ($P < 0.01$) than in spring.

The highest Cu concentration was found at the altitude of 550 m and the lowest at the altitude of 800 m (17.04 ± 3.24 µmol L^{-1}vs. 14.37 ± 2.31 µmol L^{-1}). Zn levels were higher at the altitudes of 950 m and 800 m

Table 4. Percentage of eosinophiles and phagocytic activity of sheep blood cells.

Factor	Eosinophiles (%)			Phagocytic activity (%)		
	N	$\bar{x} \pm$ SD	Significance	N	$\bar{x} \pm$ SD	Significance
Altitude 1	89	9.26 ± 7.62	**	69	85.04 ± 13.06	***
2	44	5.81 ± 5.10	1 : 2**	16	92.56 ± 7.57	1 : 3**
3	43	8.77 ± 6.15		33	95.07 ± 7.39	
Season 1	87	7.77 ± 6.78	NS	36	84.24 ± 14.93	**
2	89	8.78 ± 6.89		78	90.90 ± 9.81	
Year 1	88	7.80 ± 6.68	NS	57	88.40 ± 12.57	NS
2	88	8.68 ± 6.98		57	89.27 ± 11.49	

For explanations see Table 2. Interactions: Altitude*Season ($P < 0.01$); Season*Year ($P < 0.05$).

Table 5. Concentration of copper and zinc in sheep blood plasma.

Factor	Copper (µmol L^{-1})			Zinc (µmol L^{-1})		
	N	$\bar{x} \pm$ SD	Significance	N	$\bar{x} \pm$ SD	Significance
Altitude 1	89	17.04 ± 3.24	***	89	14.77 ± 3.86	***
2	44	14.37 ± 2.31	1 : 2***	44	17.00 ± 4.38	1 : 3***
3	43	14.49 ± 2.81	1 : 3*	43	17.81 ± 6.61	
Season 1	87	15.47 ± 3.36	NS	87	15.09 ± 4.62	NS
2	89	16.00 ± 3.00		89	16.63 ± 5.13	
Year 1	88	16.39 ± 3.48	NS	88	15.97 ± 5.61	NS
2	88	15.17 ± 2.82		88	15.75 ± 4.28	

For explanations see Table 2. Interactions: Altitude*Season ($P < 0.05$); Altitude*Season ($P < 0.01$).

a.s.l. than at the altitude of 550 m a.s.l. (17.81 ± 6.61 µmol L^{-1}, 17.00 ± 4.38 µmol L^{-1} vs. 14.77 ± 3.86 µmol L^{-1}) (Table 5). Significant interactions in Cu and Zn concentrations were recorded between Altitude*Season ($P < 0.05$, $P < 0.01$).

Discussion

Blood sampling is a powerful tool to address physiological responses of an animal and can indicate important information on its health, welfare and nutritional state. The examination of blood provides the opportunity to clinically investigate the presence of several metabolites and other constituents in the body of sheep. It plays a vital role in the physiological, nutritional and pathological status of an organism. Many parameters can be accessed via a blood sample, such as hormonal and immune status, and metabolic levels. However, the objective of the study was to evaluate hematological parameters and trace microminerals in blood plasma concentrations in sheep kept in a highland border region of the Czech Republic, where sheep can be grazed on pastures deficient in trace elements.

The highest haemoglobin concentration in sheep blood was recorded at the altitude of 950 m. The effect of altitude on erythrocytic values has been studied by many investigators. It is now a well established fact that reduced oxygen pressure in highland regions leads to an increased production and release of erythropoietin, thereby stimulating erythropoiesis as a coping or adaptive mechanism to low oxygen levels in such environments (Jessen et al. 1991; Storz & Moriyama 2008). Therefore, the higher haemoglobin and haematocrit values of sheep bred at the altitude of 950 m a.s.l. could provide evidence of the adaptation of these animals to a lower amount of atmospheric oxygen (Herrera et al. 2007).

The highest number of leucocytes was detected also at the altitude of 950 m. In addition, higher leucocyte counts were recorded in spring. Few sources provide scarce data concerning optimal levels of white blood cell markers in sheep kept in highlands. However, the presence of haemoglobin with a high oxygen affinity helps the animals to adapt to high altitudes (Weber 2007). We can only surmise that the higher leukocyte count was caused by adaptation.

Eosinophile percentages were highest in the blood of ewes at the altitude of 550 m, but values at the other altitudes were not low enough to be deemed typical symptoms of stress. Phagocytosis is an important mechanism of nonspecific immunity in which an antigen is presented to other immune cells for an initiation of the specific immune response. The highest value of phagocytic activity of sheep blood cells was recorded at the altitude of 950 m and the lowest one at the altitude of 550 m. This may be attributed to a better adaptation of the organism in a mountainous environment (Dacie 1991). There were also higher phagocytic activities in fall and in sheep bred under the ecological system.

The highest Cu concentration was found at the altitude of 550 m a.s.l. Cu content in the plasma of sheep studied was not marginal. We did not find Cu deficient farms with an exception of the October 2005 observation (1.18 mg kg^{-1} in dry matter) (Table 1) at the altitude of 550 m. The higher Cu intake could be caused by the extraordinary consumption of mineral licks. Mineral supplementation in the diet is almost ubiquitous in lactating ewes, 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, with both likely to be affected by interactions with other food constituents (Massanyi et al. 2003a). Hence, trace elements deficiencies are often suspected by veterinary surgeons in low performance herds, and when assessed, a deficient status is considered as the likely cause of disorders. However, most of these herd disorders have a multifactorial origin, and the importance of individual trace mineral deficiencies as risk factors has not been evaluated in commercial herds (Slavik et al. 2006).

Even though we did not record toxic values and the concentrations measured for plasma Cu were in the range used by veterinary laboratories (Laven & Smith 2008), animals in some areas could be potentially exposed to environmental contaminants (Kottferova & Korenekova 1995; Jančová et al. 2006). However, sheep are more sensitive to copper supplementation compared to other species. Zn levels in blood plasma were similar to those reported in most other studies.

In comparison to Cu plasma concentration, Zn levels were on the opposite end of the spectrum. The lowest Zn content in blood plasma was at the altitude of 550 m, although dry matter feed contained the highest amount of Zn (61.7 mg kg^{-1}) (Table 1). The content of microelements Cu and Zn per 1 kg of dry matter of fodders was similar to the recommendation by Nutrient Requirements of Sheep (1985) and Sommer et al. (1994) standards.

The Zn concentration was lower in spring than in fall. Industrial and agricultural activity has also resulted in increased environmental concentrations of trace metals, such as Cu and Zn, in certain areas. These are essential elements; deficient intake results in impairment of biological functions but these metals are toxic when ingested in excess (Lopez et al. 2000). The toxic effects of risk elements on sheep may be manifested via health disturbances and a decreased performance and reproductive indices (Kottferova & Korenekova 1997; Bires et al. 1997). However, we did not record any health problems in the observed animals.

The study found significant differences in multiple parameters among the observed sheep. We can conclude that hematological markers and trace mineral content in grazing sheep may be influenced by the altitude and season.

Acknowledgements

This work was carried out during the realization of the project "CEGEZ No. 26220120042" supported by the Operational Programme Research and Development funded by the European Regional Development Fund and also supported by the Ministry of Education, Youth and Sports of the Czech Republic, grant No. MSM 6007665806 and by the Slovak Research and Development Agency for project SK-CZ-0021-09/Contact MEB 0810046.

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Received January 16, 2010
Accepted October 5, 2010