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EFFECT OF DIFFERENT ENVIRONMENTAL FACTORS ON SELECTED BLOOD MINERALS IN SHEEP

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ABSTRACT

Aim of this study was to confirm the hypotheses that calcium, phosphorus, copper and zinc contents in blood plasma of sheep are influenced by altitude, season, breed, management system and herd. The ewes were kept in three herds, at two systems, and at four altitudes. Blood samples were divided according to following factors: Altitude (550 m, 800 m, 910 m, 950 m above sea level), Season of the year (spring 2004, autumn 2004, spring 2005, autumn 2005), Season (spring, autumn), Breed (Merinolandschaf, Charolais, Sumavian sheep), System (ecological, non-ecological), and Herd (three herds). Concentration of phosphorus was significantly increased in altitude of 800 m above sea level (2.46 mg.l^{-1}). Significant differences were recorded within the factors Season of year and Season, due to lower levels of calcium in the autumn 2004 (2.27 mg.l^{-1}) and phosphorus in the spring 2005 (1.36 mg.l^{-1}). The highest concentrations of phosphorus were recorded within the factors Breed and Herd, as a result of Merinolandschaf [or herd 1] (2.46 mg.l^{-1}). Higher levels of phosphorus were found in non-ecological system (1.77 mg.l^{-1} vs. 1.36 mg.l^{-1}). The copper and zinc concentrations were affected by the factor Altitude (P<0.001). The lowest level of copper was recorded in the spring 2004 (11.65 µmol.l^{-1}); between spring and autumn (factor Season) a significant difference was recorded in zinc (15.08 µmol.l^{-1} vs. 17.00 µmol.l^{-1}; P<0.01). The highest value of copper was measured in the Charolais sheep (17.64 µmol.l^{-1}), the highest concentration of zinc in the Sumavian sheep (17.34 µmol.l^{-1}) and the lowest one in the Charolais sheep (14.68 µmol.l^{-1}; P<0.01). The higher copper concentration (16.14 µmol.l^{-1} vs. 14.49 µmol.l^{-1}; P<0.01) and the lower zinc concentration (17.81 µmol.l^{-1} vs. 15.22 µmol.l^{-1}; P<0.01) was measured in non-ecological system. Significant differences in both microminerals (cooper and zinc) were found among herds. Results of this study confirm variable factors affecting concentration of mineral elements in the blood.

Key words: sheep, calcium, phosphorus, copper, zinc

INTRODUCTION

In order to provide a high quality sheep production a more information is needed on their mineral requirements in relation to management system. Knowing the microelement status of the different sheep categories and interpreting test results correctly is of major importance for determining potential preventive arrangements. More than twenty minerals are considered to be nutritionally essential to the animal. Depending on the quantities required by the animal, they can be grouped into major minerals and trace elements. Of the major minerals the most important are calcium and phosphorus. Most of the major minerals and trace elements are widely distributed in the herbage and the other feeds eaten by the animal, and occur in sufficient quantities to meet animal requirements. Phosphorus is an essential nutrient and is involved, as phosphate, in most of the metabolic activities of the body, as well as in bone formation (Kebreab and Vitti, 2005). Decreased growth and milk production, poor conception and depraved appetite are the general symptoms of calcium and phosphorus deficiency. Animals, therefore, need an adequate supply of both minerals for optimum growth and production (Vitti et al., 2005).

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The important trace elements are copper and zinc, except for cobalt, iron, iodine, manganese and selenium, of course. Copper has a basic role in the metabolism and transition of iron in the body. It is an essential component of several enzymes such as ceruloplasmin, cytochrome C oxidase, lysil oxidase, superoxid dismutase, and tyrosinase, that are required to maintain host homeostasis (Swenson and Reece, 2004). Deficiency has been linked to a variety of clinical signs, including anemia, pale coat, spontaneous fractures, poor capillary integrity, myocardial degeneration, hypomyelinization of the spinal cord, impaired reproductive performance, and decreased resistance to infectious disease (Nutrient Requirements of Sheep, 1985; Underwood and Suttle, 2001; Heidarpour Bami et al., 2008).

Zinc is important for the synthesis of proteins and nucleic acids, a number of catalytic, structural and regulatory functions, formation of skin structures and skeleton metabolism. In ruminants, it plays an important role in rumen fermentation processes, mainly cellulose digestion and formation of volatile fatty acids (Cao et al., 2000; O'Dell, 2000; Massanyi et al., 2001; Korenekova et al., 2006).

In sheep nutrition, a precise balance of daily rations, particularly on minerals, is necessary but not often fulfilled. The content of minerals in different pasture plant fodders is diversified. Therefore, their deficiency or imbalance in the diet could lead to disturbances of metabolic function of organism (Šrejberová et al., 2008). Heavy metal contamination of plants would be dangerous for sheep, because sheep seem to be most sensitive especially to an increased intake of copper. Elgervi et al. (1999) showed findings resulting from copper intoxication in Valachian sheep that animals reared under such conditions were adapted to a certain degree to chronic intake of increased doses of Cu.

The aim of the study was to test the hypotheses that calcium, phosphorus, copper and zinc contents in blood plasma of sheep are influenced by the altitude, season, breed, management system and herd. We determined blood plasma concentrations of macrominerals and microminerals in sheep in selected herds kept in a mountainous region.

MATERIALS AND METHODS

The adult female sheep (2-4 years) were kept in three herds, at two systems, and four altitudes. Blood samples were divided according to factors: Altitude (A1 – 550 m, n=96; A2 – 800 m, n=48; A3 – 910 m, n=35; A4 – 950 m, n=12 above sea level), Breed (B1 – Charolais, n= 85; B2 - Merinolandschaf, n= 48; B3 – Sumavian sheep, n= 58), System (S1 – ecological with majority of Sumavian sheep, n=47, S2 - non-ecological, n=144), Herd (H1 – n=48; H2 – n=96; H3 – n=47). The Herd 1 was formed with 81 % of Merinolandschaf breed, 12 % of Merino sheep and 7 % of Bergschaf breed; The Herd 2 contained 79 % of Charolais breed and 21 % of Suffolk breed; The Herd 3 had 91 % of Sumavian sheep, 5 % Cigaya sheep and 4 % crossings.

Blood samples were collected during spring and autumn within two years. They were divided according to the season of the year into four groups: Seas Year 1 (spring 2004; n=36), Seas Year 2 (autumn 2004; n=34), Seas Year 3 (spring 2005; n=61), Seas Year 4 (autumn 2005; n=60). Other climatic factor was the Season; blood samples were divided according to two periods: Season 1 (spring; n=97) and Season 2 (autumn; n=94).

The observations were done at the South-west region of Czech Republic. Pasture (grazing-land, grassland) is based on granodiorite geological footwall of weinsberg type in the internal zone of the South Bohemian Massif. In this region sheep are mostly raised on smaller farms. The animals were fed on pasture grass and they had *ad libitum* access to water and free-choice mineral supplement. Mineral supplementations were provided at a certain location in each pasture in free-choice mineral feeders. Feed intake was monitored daily in each observation for 3 days. Feed samples were dried and ground in a mill to pass through a 1 mm mesh screen. In summer, all the animals grazed only without additional feeding. The amount and source of macro- and microelements in daily rations for sheep are presented in Table 1. The sheep were in good health condition (including clinical state of the udder) during the whole experiment.

Table 1: Consumption feeds and mineral content in dry matter per day

Herd	Season	Pasture (kg)	Hay (kg)	Oats (kg)	Copper ($mg.kg^{-1}$)	Zinc ($mg.kg^{-1}$)
1	SY1	1.82	-		7.21	57.12
	SY2	1.18	0.93		4.33	51.04
	SY3	2.29	-		9.14	64.52
	SY4	1.33	0.64		1.82	74.10
2	SY1	1.75	1.90	0.20	4.38	62.00
	SY2	0.97	1.28	-	3.26	59.60
	SY3	1.52	1.76	0.18	5.37	35.52
	SY4	1.28	1.43	-	4.04	38.68
3	SY1	2.17	-		5.97	29.06
	SY2	1.03	0.82		4.30	66.90
	SY3	1.50	0.62		9.53	32.10
	SY4	1.03	1.10		8.65	63.57

SY1=spring 2004, SY2=autumn 2004, SY3=spring 2005, SY4=autumn 2005

General health parameters were recorded for each of three days of the 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 concentration of calcium, phosphorus, 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). The normal distribution of the data was evaluated by Wilk-Shapiro/Rankin Plot procedure. Data consisting of calcium, phosphorus, copper and zinc in blood plasma conformed to a normal distribution. Among-group comparisons were analysed using a General linear model ANOVA (General AOV/AOCV). Values are expressed as means ± S.E. Significant differences among means were tested by Bonferroni´s test.

RESULTS

Mean concentrations of phosphorus significantly differed in the factor of Altitude (Table 2). Level of this mineral was higher in altitude of 800 m above sea (1.43 ± 0.06 $mg.l^{-1}$, 2.46 ± 0.09 $mg.l^{-1}$, 1.43 ± 0.11 $mg.l^{-1}$, 1.17 ± 0.18 $mg.l^{-1}$). Significant differences were observed in the factor Season of year in consequence of lower levels of calcium in the autumn 2004 (2.27 ± 0.09 $mg.l^{-1}$) and phosphorus in the spring 2005 (1.36 ± 0.08 $mg.l^{-1}$), also between spring and autumn (factor Season).

Values of phosphorus were different in factors Breed and Herd, as a result of higher concentrations of Merinolandschaf or Herd 1 (2.46 ± 0.09 $mg.l^{-1}$). Significantly higher levels of phosphorus were found in non-ecological system (1.77 ± 0.06 $mg.l^{-1}$ vs. 1.36 ± 0.11 $mg.l^{-1}$).

We found interactions in calcium level between Season of year*System (P<0.001); Season*Breed (P<0.001); Season*System (P<0.05) and Season*Herd (P<0.001). In case of phosphorus the interactions between Season of year*System (P<0.01); Season*Breed

Table 2: Concentrations of macrominerals in blood plasma

Factor	Calcium ($mg.l^{-1}$)				Phosphorus ($mg.l^{-1}$)			
	N	$\bar{x}$ ± SE	p		N	$\bar{x}$ ± SE	p	
Altitude - 1	96	2.46 ± 0.06	0.6316		96	1.43 ± 0.06	0.0000***	1:2***
- 2	48	2.46 ± 0.08			48	2.46 ± 0.09		
- 3	35	2.52 ± 0.09			35	1.43 ± 0.11		2:3,4***
- 4	12	2.68 ± 0.16			12	1.17 ± 0.18		
Seas Year-1	36	2.67 ± 0.09	0.0016**	2:3**	36	1.68 ± 0.10	0.0005***	3:4***
- 2	34	2.27 ± 0.09		1:2*	34	1.55 ± 0.11		
- 3	61	2.71 ± 0.07			61	1.36 ± 0.08		
- 4	60	2.45 ± 0.07			60	1.89 ± 0.08		
Season - 1	97	2.64 ± 0.06	0.0013**		97	1.63 ± 0.06	0.0076**	
- 2	94	2.37 ± 0.06			94	1.89 ± 0.06		
Breed - 1	85	2.44 ± 0.06	0.3485		85	1.39 ± 0.07	0.0000***	1:2***
- 2	48	2.51 ± 0.08			48	2.46 ± 0.09		2:3***
- 3	58	2.58 ± 0.07			58	1.43 ± 0.08		
System - 1	47	2.57 ± 0.08	0.3267		47	1.36 ± 0.11	0.0022**	
- 2	144	2.47 ± 0.05			144	1.77 ± 0.06		
Herd - 1	48	2.51 ± 0.08	0.5477		48	2.46 ± 0.09	0.0000***	1:2,3***
- 2	96	2.46 ± 0.06			96	1.43 ± 0.07		
- 3	47	2.57 ± 0.08			47	1.36 ± 0.09		

*P<0.05; **P<0.01; ***P<0.001
Altitude: 1=550 mnm, 2= 800 mnm, 3= 910 mnm, 4= 950 mnm
Seas Year = Season of Year: 1=spring 2004, 2=autumn 2004, 3=spring 2005, 4=autumn 2005
Season: 1=spring, 2=autumn
Breed: 1=Charolais, 2=Merinolandschaf, 3=Sumavian sheep
System: 1=ecological (majority of Sumavian sheep), 2=non-ecological
Herd: 1= majority of Merinolandschaf, 2= majority of Charolais, 3= majority of Sumavian sheep

Interactions:
Calcium - Season of Year*System=0.0004***; Season*Breed= 0.0000***; Season*System=0.0297*; Season*Herd=0.0006***
Phosphorus - Season of Year*System=0.0088**; Season*Breed= 0.0000***; Season*System=0.0147*; Season*Herd=0.0000***

(P<0.001); Season*System (P<0.05); Season*Herd (P<0.001) were found.

The highest copper concentration (Table 3) was in the Altitude 1 and the lowest in the Altitude 4 (17.07 ± 0.25 μmol.l^{-1} vs. 10.88 ± 0.71 μmol.l^{-1}). Zinc levels were higher in Altitudes 2 and 3 than in 1 and 4 (17.53 ± 0.64 μmol.l^{-1}, 18.99 ± 0.76 μmol.l^{-1} vs. 14.77 ± 0.46 μmol.l^{-1} , 14.94 ± 1.28 μmol.l^{-1}). Differences among altitudes were highly significant in both parameters (Table 3).

The lowest level of copper in factor Season of the year was in the spring 2004 (11.65 ± 0.41 μmol.l^{-1}) and differences with other parameters were significant (P<0.001). The greatest concentrations of zinc were noted between the autumn 2004 and the spring 2005 (19.34 ± 0.77 μmol.l^{-1} vs. 14.18 ± 0.58 μmol.l^{-1}) (P<0.001). Also, between spring and autumn in the factor Season a significant difference was recorded (15.08 ± 0.49 μmol.l^{-1} vs. 17.00 ± 0.49 μmol.l^{-1}) (P<0.01).

Factor Breed had significant effect on both microminerals (Table 3). The highest value of copper was measured in the Charolais sheep (17.64 ± 0.30 μmol.l^{-1}) (P<0.001) and the highest concentration of zinc in the Sumavian sheep (17.34 ± 0.63 μmol.l^{-1}) and the lowest in the Charolais sheep (14.68 ± 0.53 μmol.l^{-1}) (P<0.01).

Factor System had significant effect on copper (14.49 ± 0.46 μmol.l^{-1} vs. 16.14 ± 0.26 μmol.l^{-1}) (P<0.01) and zinc level (17.81 ± 0.71 μmol.l^{-1} vs. 15.22 ± 0.40 μmol.l^{-1}; P<0.01). The highest value of copper concentration was recorded in the Herd 2 (17.04 ± 0.30 μmol.l^{-1}). Level of zinc was lowest in the Herd 2 and highest in the Herd 3 (16.10 ± 0.69 μmol.l^{-1}; 14.77 ± 0.49 μmol.l^{-1}; 17.81 ± 0.71 μmol.l^{-1}; P<0.01).

Significant interactions were noted within the factors Season of Year*System (P<0.01); Season*Breed (P<0.01) and in case of zinc within the factors Season of Year*System (P<0.01).

DISCUSSION

The aim of the study was to determine blood plasma concentrations of macrominerals and microminerals in sheep in selected herds kept in a mountainous region. This part of Czech Republic is a border region and sheep are reared extensively there, mainly in pastures deficient in trace elements.

In this study concentration of phosphorus was significantly higher in altitude of 800 m above sea. Evaluating the season of the year the differences were

Table 3: Concentrations of microminerals in blood plasma

Factor	Copper (μmol.l^{-1})				Zinc (μmol.l^{-1})			
	N	$\bar{x}$ ± SE	P		N	$\bar{x}$ ± SE	P	
Altitude - 1	94	17.07 ± 0.25	0.0000***	1:2,4***	94	14.77 ± 0.46	0.0000***	1:3***
- 2	48	13.58 ± 0.35		2:3***	48	17.53 ± 0.64		3:4*
- 3	34	15.83 ± 0.42		3:4***	34	18.99 ± 0.76		1:2*
- 4	12	10.88 ± 0.71			12	14.94 ± 1.28		
Seas Year-1	36	11.65 ± 0.41	0.0000***	1:2,3,4***	36	16.64 ± 0.74	0.0000***	2:3***
- 2	33	15.44 ± 0.43			33	19.34 ± 0.77		2:4**
- 3	59	15.33 ± 0.32			59	14.18 ± 0.58		
- 4	60	14.93 ± 0.32			60	16.07 ± 0.57		
Season - 1	95	15.37 ± 0.28	0.8937		95	15.08 ± 0.49	0.0072**	
- 2	93	15.42 ± 0.28			93	17.00 ± 0.49		
Breed - 1	82	17.64 ± 0.30	0.0000***	1:2,3***	82	14.68 ± 0.53	0.0063**	1:3*
- 2	48	14.37 ± 0.39			48	16.09 ± 0.69		
- 3	58	14.17 ± 0.36			58	17.34 ± 0.63		
System - 1	46	14.49 ± 0.46	0.0021**		46	17.81 ± 0.71	0.0018**	
- 2	142	16.14 ± 0.26			142	15.22 ± 0.40		
Herd - 1	48	14.37 ± 0.42	0.0000***	1:2,3***	48	16.10 ± 0.69	0.0023**	2:3**
- 2	94	17.04 ± 0.30		2:3*	94	14.77 ± 0.49		
- 3	46	14.49 ± 0.43			46	17.81 ± 0.71		

*P<0.05; **P<0.01; ***P<0.001

Altitude: 1=550 mnm, 2= 800 mnm, 3= 910 mnm, 4= 950 mnm
Seas Year = Season of Year: 1=spring 2003, 2=autumn 2003, 3=spring 2004, 4=autumn 2004
Season: 1=spring, 2=autumn
Breed: 1=Charolais, 2=Merinolandschaf, 3=Sumavian sheep
System: 1=ecological (majority of Sumavian sheep), 2=non-ecological
Herd: 1= majority of Merinolandschaf, 2= majority of Charolais, 3= majority of Sumavian sheep

Interactions:
Copper - Season of Year*System=0.0025**; Season*Breed= 0.0018**
Zinc - Season of Year*System=0.0098**

caused by various levels of calcium and phosphorus in the feed. Due to grazing there were some changes in protein content and protein fraction in localities with different altitudes between 450 and 900 metres above sea level (Rajčáková et al., 2003). The differences in macromineral concentrations in the factors Breed and Herd were probably due to the effect of the system type (ecological or non-ecological). Higher levels of phosphorus were found in non-ecological system. Grass and herbage are the most natural and optimal feedstuff for sheep. Grazing management should notably regulate the pasture composition and decrease occurrence of weed and less value strain of gramineous grasses (Rajčáková et al., 2003; Čermák et al., 2006).

The copper and zinc concentrations were affected by the altitude of the farm and the season. The highest value of copper was measured in the Charolais sheep, the highest concentration of zinc in the Sumavian sheep and the lowest in the Charolais sheep. There was higher copper concentration and lower zinc concentration in non-ecological system. However, copper levels in sheep were generally high in this place (Breed 1, Herd 2). Even though toxic values were not recorded and the concentrations measured for plasma copper were in the range used by veterinary laboratories (Bock und Polach, 1994; Laven and Smith, 2008), animals in some areas could be potentially exposed to environmental contaminants (Kottferova and Korenekova, 1995; Massanyi et al., 2003).

Chemical and mainly metallurgical industry belong to the main source of pollution of air as well as environment with metals. The negative impact of the emission is visible in all branches of agricultural production. Transition metals at trace levels play an important role in animal body functions. The amount of an element which accumulates in the organs depends on the exposure time, the quantity ingested, the production and reproduction phases of the animals, as well as their age and breed (Kottferova and Korenekova, 1997). Sub-lethal exposure to these elements can result in adverse effects on a variety of physiological and biochemical processes. However, a number of factors, such as breed, diet, and the concentration of copper antagonists (Fe, S, and Mo) may affect responses of sheep to copper (Solaiman et al., 2006). Industrial and agricultural activity has also resulted in increased environmental concentrations of trace metals, such as copper and zinc, 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)

However, in case of the essential trace elements (copper and zinc), there was no evidence of deficiencies in sheep in observed herds. Zinc levels in blood plasma were similar to those reported in most other studies.

The seasonal variations can change the concentrations of macrominerals in forages. Therefore, our results of variable impact of seasonal changes on the concentration of minerals in the blood suggest the need for supplementation of deficient minerals like Cu, Zn in the forms which are readily available and affordable by farmers for optimal productivity. Copper and zinc liver levels change progressively with soil levels, and the pattern is especially marked for copper (Lopez et al., 2002). The content of macroelements calcium and phosphorus, and microelements copper and zinc per 1 kg of dry matter of fodders was similar to the standards recommendation from Nutrient Requirements of Sheep (1985), Vrzgula et al. (1990), and Sommer et al. (1994).

The results of this study show that in order to achieve an adequate or marginal zinc status in observed ewes it is sufficient for them to receive Zn from mineral lick. However, the pursuit of preventive diagnostic tests in herds is important for the assessment of the herd health status.

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