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Changes of macromineral and trace element concentration in the blood of ewes during lactation period

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Abstract: The aim of the present research was to determine the changes in macromineral and trace element concentrations in ewes' blood during the lactation period. Thirty-six Travnik pramenka ewes at the average age of 3–5 years were included in research and they were evaluated at three stages of lactation: early stage (40 ± 5 days of lactation), medium stage (80 ± 5 days of lactation) and late stage (120 ± 5 days of lactation). In ewes' blood concentrations of macroelements (Ca, P, K, Na, Mg) and trace elements (Fe, Cu, Zn, Mo, Co, Se, Cd, As) were determined. The average concentrations of macroelements and trace elements in the blood of ewes during lactation were mostly within the reference values. Se concentrations in blood were close to the deficient ones, while concentrations of Cd and As were negligible. Concentration of Ca in blood was the lowest in the early lactation stage, higher in the late stage, and the highest in the medium stage (88.25 mg/l, 96.43 mg/l and 103.43 mg/l, respectively). A significant increase in the concentrations of Mg, Co and Cd and a significant decrease in the concentrations of Na, Fe, Cu, Zn, Mo and Se were found in blood with lactation progress. Significant correlations between the studied parameters were found, especially between macroelements (Ca : K, P : Na, K : Na, K : Mg). Positive correlations were found between some macroelements and trace elements (Ca : Co, Ca : Cd, K : Co, Na : Zn, Na : Se) and between trace elements (Fe : Cu, Cu : Zn, Cu : Mo, Cu : Se, Zn : Se, Mo : Se and Co : As). Negative correlations were found between Ca and Cu, Ca and Mo, P and Cd, K and Cu. Obtained results may help in monitoring the nutritional status and health of ewes during the lactation period, providing a clearer idea related to the supply of macrominerals and trace elements to ewes. Besides, results indicated that the environment of the farming area was not polluted with toxic elements.

Keywords: essential elements; heavy metals; blood serum; lactating sheep

Travnik sheep are originally from Bosnia and Herzegovina while in Croatia they are mostly kept in the area of Bilogora. It is a late maturing breed, physically developed within the third and the fourth year, with very good fertility and production of 70 l to 130 l of milk (Novoselec et al. 2020). These sheep are reared in the extensive rearing system; thus, it is hard to assess the consumption of pasture for each

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animal. Therefore, blood sampling presents a reliable way of determining macromineral and trace element supply in ewes.

Lactation, along with pregnancy, is a very demanding period for the animal which is considered to modify metabolism in animals. Then, ewes need high intake of protein, energy and minerals. The concentrations of most macrominerals and trace elements in blood have been scarcely investigated in lactating sheep. Blood sampling is a powerful tool to address the physiological response of an animal which may indicate important information on its health, welfare and nutritional state (Antunovic et al. 2017a). Blood samples are usually used to determine the status of macrominerals and trace elements and present a minimally invasive procedure (Herdt and Hoff 2011). Although blood samples (serum or plasma) are not always the best indicator for detection of the marginal status of trace elements, possibly due to the activation of trace element storage in the body which compensates their deficiency, thus clinical signs cannot be visible (Ramirez-Perez et al. 2000). Macroelements (Ca, P, Mg, Cl, K, Na and S) are required in large quantities while microelements or trace elements (Co, Cu, I, Fe, Mn, Mo, Se and Zn) are required in smaller quantities. The trace elements depend on nutrition and their deficiency or disturbances in metabolism are relatively common, while substantial information is available about their metabolism and the amounts needed for optimum health and productivity in animals (Herdt and Hoff 2011). The influence of various factors (nutrition, bioavailability of minerals, different interactions of elements, antagonisms and synergisms, reproductive status, breed, species, age, sex, environmental impact, lab work etc.) on the movement of concentrations of macrominerals and trace elements in the blood of animals is significant. Excessive mineral supplies pollute the environment and the chosen or default policy of mineral nutrition on a given farm leaves a "mineral footprint" (Suttle 2010). Kovacik et al. (2017) found that heavy metal levels significantly contribute to environmental contamination, among which Cd, Cu, Hg, Pb and Zn are the most notable. Heavy metal contamination can be transferred to animals through direct exposure, polluted water, and crops grown on irrigated sewage, industrial effluents, vehicle emission and dirty slaughter houses (Jukna et al. 2006). The knowledge regarding normal values of minerals and trace elements in the blood of different animals is of academic and practical importance for clinical and experimental interpretations (Broucek et al. 2009), as well as regarding metabolism and health monitoring of the ewes (Antunovic et al. 2017b).

Based on these previous results, we hypothesised that the concentrations of macrominerals and trace elements in ewes' blood would fluctuate during lactation. Therefore, the aim of the present study was to investigate changes in a higher number of macrominerals and trace elements in ewes' blood during different stages of lactation.

MATERIAL AND METHODS

Analyses of macrominerals and trace elements in blood were conducted with 36 Travnik ewes at an average age of 3–5 years in an extensive production system. Blood was taken from the same ewes in three stages of lactation: early stage (40 ± 5 days of lactation), medium stage (80 ± 5 days of lactation) and late stage (120 ± 5 days of lactation). These ewes were selected from the herd of 1 000 animals. The selected ewes were healthy and in a good physical condition. Ewes were grazing on extensive pastures all day. Water and mineral lick were offered to ewes *ad libitum*. The present study was carried out in 2019, when ewes were in the third lactation with single lamb in litter. The family sheep farm was located in Velika Peratovica 10 km from Grubisno polje (in Croatia, 45°45'25''N, 17°14'51''E, ~211 m above sea level). Feed samples (green forage from pastures) were dried and ground into a fine powder using a heavy metal-free ultra centrifugal mill (Retsch ZM 200) or knife mill (GM 200).

From each ewe during different stages of lactation period, blood samples were collected from the jugular vein (10 ml) into the sterile vacuum tubes Venoject® (Sterile Terumo Europe, Leuven, Belgium). Afterwards, blood samples were centrifuged at 1 609.92 *g* within 10 min to obtain serum. In serum samples concentrations of macrominerals (calcium, inorganic phosphorus, potassium, sodium and magnesium) were determined and expressed in mg/l, while concentrations of trace elements (iron, copper, zinc, molybdenum, cobalt, selenium, cadmium and arsenic) were expressed in µg/l.

Feed and blood serum samples were digested with 10 ml of a 5 : 1 mixture of HNO_3 and H_2O_2 at 180 °C for 60 min in a microwave oven (Mars 6; CEM

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Corporation, Matthews, NC, USA). The concentrations of macrominerals and trace elements in solutions of digested feed, water and blood serum were determined by inductively coupled plasma (ICP; Optima 2100 DV; PerkinElmer Inc., Wellesley, MA, USA). Each batch of all samples run on the ICP was analysed with an internal pooled plasma control and with the reference material prepared in the same way as were all the other samples. All samples were analysed in duplicate. Macromineral and trace element contents of feed and water of ewes (mg/kg dry matter) are presented in Table 1.

Bioethics Committee for Research on Animals of the Faculty of Agrobiotechnical Sciences Osijek established that the present research was carried out under the legal provisions according to Animal Protection Act (NN 133/06, NN 37/13 and NN 125/13). Animal handling practices met the requirements of the Directive 2010/63/EU about the protection of animals used for the experiment (EU 2010).

The distribution of data was verified using the Shapiro-Wilk test (PROC UNIVARIATE). Results were expressed as mean values, standard deviation and standard error of the mean estimated by MEANS procedure. Analysis of variance was performed by GLM procedure to analyse the effect of lactation stage, while differences between groups were determined by Tukey's test at a level of $P < 0.05$. Additionally, Pearson's correlation coefficient between macro and trace elements in the blood of ewes during lactation was estimated by CORR procedure. All data were analysed with the statistical software SAS v9.4® (SAS Institute Inc., Cary, NC, USA). Reference values for blood macrominerals and trace elements from the laboratory were compared with selected reference values (Puls 1994; Underwood and Suttle 1999; Herdt and Hoff 2011; Schweinzer et al. 2017).

Table 1. Macromineral and trace element contents (mg/kg dry matter) of green forage (pasture) and water

Parameters	Green forage			Water
	1	2	3	
Ca	8 870.84	7 261.10	6 986.36	121.9
Mg	3 395.83	1 869.26	2 755.74	56.48
K	44 769.84	39 178.03	49 822.06	< LD
P	5 748	5 857	5 100	< LD
Na	104.36	40.96	205.55	5.50
Cu	10.29	8.01	7.21	0.002
Fe	274.30	226.60	207.90	< LD
Zn	33.92	28.07	29.99	< LD
Mo	1.070	3.031	0.771	ND
Co	0.108	0.083	0.109	0.003
Se	0.052	0.047	0.066	< LD
Cd	< LD	< LD	0.037	< LD
As	< LD	< LD	< LD	< LD

LD = instrumental detection limits in mg/kg (Ca: 3.259; Mg: 0.007; K: 1.468; P: 0.164; Cu: 0.000 2; Fe: 0.000 18; Zn: 0.064 4; Se: 0.002 2; Cd: 0.001 58, As: 0.001 99); ND = not determined

Table 2. Descriptive statistics of macroelement concentration (mg/l) in the blood of lactating ewes

	Mean	Min.	Max.	SD	SEM	Reference values*
Ca	96.01	70.15	114.63	10.07	0.97	78–124
P	106.45	64.42	138.30	13.81	1.34	40–80[1]
K	208.18	158.36	262.94	20.67	2.00	187–371
Na	3 486.36	3 218.90	4 005.99	114.17	11.04	3 220–3 335[2]
Mg	24.80	18.24	32.63	2.70	0.26	16.8–32.7

*Schweinzer et al. (2017); [1]Puls (1994); [2]Underwood and Suttle (1999)

RESULTS

The analysis of Table 2 shows that the average concentrations of macrominerals in the blood of ewes during lactation were mostly within the reference values, except for the higher P concentrations.

Table 3. Descriptive statistics of trace element concentration (µg/l) in the blood of lactating ewes

	Mean	Min.	Max.	SD	SEM	Reference values*
Fe	431.19	73.95	998.00	271.13	35.60	662–7 040
Cu	725.92	449.30	1 582.00	184.58	17.84	200–1 200
Zn	440.05	60.89	1 320.01	260.14	30.01	330–1 100
Mo	2.72	0.04	9.29	2.11	0.25	2.7–1 430
Co	0.23	0.05	1.41	0.17	0.02	0.2–2.5
Se	55.68	23.00	125.70	24.25	2.34	8.2–165.5
Cd	0.44	0.04	1.86	0.36	0.04	< 200[1]
As	2.17	0.15	4.38	0.87	0.08	< 40[1]

*Schweinzer et al. (2017); [1]Puls (1994) – determined in whole blood

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The analysis of Table 3 documents that half of the presented average concentrations of trace elements in the blood of ewes during lactation were within the reference values. Concentration of Ca in blood was the highest in the medium stage followed by the late stage and it was the lowest in the early lactation stage. Concentration of Na in ewes' blood was the highest in the early stage, compared to the later stage, while in the medium stage of lactation it did not differ significantly. As lactation progressed, the concentration of Mg in ewes' blood significantly increased in the late stage compared to the early and medium stage, while concentrations of P and K did not differ significantly in relation to lactation stage (Table 4). A significant decrease of Fe concentration in ewes' blood was found in the late stage compared to the early stage, while in the medium lactation stage it did not differ significantly. A similar decrease was observed in Cu, Zn, Mo and Se concentrations in the medium and late stage compared to the early stage of lactation (Table 5). A significantly increased concentration of Co was observed in the medium stage compared to the early and late stage, while Cd concentrations increased significantly in the medium and late stage compared to the early stage of lactation. Concentrations of As did not differ significantly under the lactation stage effect.

Table 4. Macroelement concentrations (mg/l) in the blood of ewes in different stages of lactation

	Early	Medium	Late	SEM	*P*-values
Ca	88.25^{a}	103.43^{b}	96.43^{c}	0.974	< 0.001
P	107.96	103.78	107.87	1.335	0.336
K	205.72	211.94	206.58	1.998	0.375
Na	3 526.94^{a}	3 485.23^{ab}	3 438.01^{b}	11.037	0.005
Mg	23.90^{a}	24.51^{a}	26.27^{b}	0.261	< 0.001

$^{a–c}$Values in rows with different letters differ significantly ($P < 0.05$)

Table 5. Trace element concentrations (μg/l) in the blood of ewes in different stages of lactation

	Early	Medium	Late	SEM	*P*-values
Fe	561.79^{a}	401.72^{ab}	249.43^{b}	35.601	0.002
Cu	867.06^{a}	673.45^{b}	617.22^{b}	17.844	< 0.001
Zn	630.02^{a}	350.01^{b}	320.04^{b}	25.002	< 0.001
Mo	4.10^{a}	1.68^{b}	1.30^{b}	0.245	< 0.001
Co	0.20^{a}	0.31^{b}	0.19^{a}	0.017	0.005
Se	76.67^{a}	46.33^{b}	41.42^{b}	2.344	< 0.001
Cd	0.19^{a}	0.59^{b}	0.55^{b}	0.034	< 0.001
As	2.21	2.05	2.29	0.084	0.504

^{a,b}Values in rows with different letters differ significantly ($P < 0.05$)

The analysis of Table 6 presents significant positive correlations between macroelements (Ca : K, P : Na, K : Na, K : Mg), macroelements and trace elements (Ca : Co, Ca : Cd, K : Co, Na : Zn, Na : Se) and between trace elements (Fe : Cu, Cu : Zn, Cu : Mo, Cu : Se, Zn : Se, Mo : Se and Co : As). Significant negative correlations were also found between macroelements and trace elements (Ca : Cu, Ca : Mo, P : Cd, K : Cu, Na : Cd, Mg : Cu, Mg : Se) and between trace elements (Zn : Cd, Mo : Co, Mo : Cd and Se : Cd).

DISCUSSION

The average concentrations of macroelements and trace elements in the blood of ewes during lactation were mostly within the limits of reference values (Puls 1994; Herdt and Hoff 2011; Schweinzer et al. 2017), except for the P concentration which was higher, and Fe which was lower compared to reference values. Assessment of the trace element status in the animal is much more difficult. It is necessary to consider its production cycle, the levels of stress imposed on the animal, the choice of analysis, the level of trace element antagonist as well as other trace minerals in the feed, and the nature of any supplementary feeds used (Lopez-Alonso 2012). Poppenga et al. (2012) pointed out that overlaps were reported between what would be considered adequate, marginally adequate, and deficient serum concentrations for some minerals. However, it should be noted that the present study was conducted on lactating sheep and it is known that milk excretes significant amounts of both macrominerals and trace elements (Antunovic et al. 2017b). Average Se concentration in the serum of ewes in the present study (55.68 μg/l) showed unsatisfactory supply of Se in ewes, as indicated by the concentrations of Se in the plants from pastures that were below the recommended values according to NRC (NRC 2007, 0.1–0.2 mg/kg dry matter diet). Humann-Ziehank et al. (2013) concluded that the sheep marginal serum Se status is 60–79.9 μg Se/l, deficient Se status

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Table 6. Coefficients of correlation with significant changes (*P*-values) for macro and micro elements in sheep blood

	Ca	P	K	Na	Mg	Fe	Cu	Zn	Mo	Co	Se	Cd	As
Ca	–	–	–	–	–	–	–	–	–	–	–	–	–
P	−0.312 (*P* = 0.754)	–	–	–	–	–	–	–	–	–	–	–	–
K	0.317 (*P* = 0.001)	0.104 (*P* = 0.295)	–	–	–	–	–	–	–	–	–	–	–
Na	0.179 (*P* = 0.071)	0.252 (*P* = 0.010)	0.237 (*P* = 0.016)	–	–	–	–	–	–	–	–	–	–
Mg	0.028 (*P* = 0.780)	0.031 (*P* = 0.758)	0.231 (*P* = 0.019)	0.061 (*P* = 0.543)	–	–	–	–	–	–	–	–	–
Fe	−0.52 (*P* = 0.743)	0.13 (*P* = 0.416)	−0.21 (*P* = 0.185	0.189 (*P* = 0.231)	−0.954 (*P* = 0.548)	–	–	–	–	–	–	–	–
Cu	−0.246 (*P* = 0.012)	−0.012 (*P* = 0.907)	−0.217 (*P* = 0.028)	0.144 (*P* = 0.148)	−0.471 (*P* < 0.001)	0.395 (*P* = 0.010)	–	–	–	–	–	–	–
Zn	−0.171 (*P* = 0.084)	0.145 (*P* = 0.143)	−0.064 (*P* = 0.518)	0.243 (*P* = 0.013)	−0.161 (*P* = 0.103)	0.161 (*P* = 0.308)	0.301 (*P* = 0.002)	–	–	–	–	–	–
Mo	−0.443 (*P* = 0.003)	0.732 (*P* = 0.645)	0.031 (*P* = 0.847)	0.093 (*P* = 0.558)	−0.064 (*P* = 0.685)	0.246 (*P* = 0.117)	0.469 (*P* = 0.002)	0.245 (*P* = 0.119)	–	–	–	–	–
Co	0.210 (*P* = 0.033)	0.080 (*P* = 0.423)	0.199 (*P* = 0.044)	0.180 (*P* = 0.069)	0.058 (*P* = 0.560)	−0.211 (*P* = 0.894)	−0.090 (*P* = 0.365)	0.051 (*P* = 0.611)	−0.394 (*P* = 0.010)	–	–	–	–
Se	−0.114 (*P* = 0.251)	0.059 (*P* = 0.557)	−0.003 (*P* = 0.974)	0.363 (*P* < 0.001)	−0.202 (*P* = 0.041)	0.203 (*P* = 0.197)	0.432 (*P* < 0.001)	0.491 (*P* < 0.001)	0.421 (*P* = 0.006)	0.057 (*P* = 0.569)	–	–	–
Cd	0.290 (*P* = 0.003)	−0.195 (*P* = 0.048)	0.011 (*P* = 0.914)	−0.298 (*P* = 0.002)	0.004 (*P* = 0.968)	−0.101 (*P* = 0.527)	−0.174 (*P* = 0.079)	−0.312 (*P* = 0.01)	−0.374 (*P* = 0.015)	0.084 (*P* = 0.397)	−0.291 (*P* = 0.003)	–	–
As	−0.128 (*P* = 0.198)	0.040 (*P* = 0.688)	−0.084 (*P* = 0.402)	−0.005 (*P* = 0.964)	0.031 (*P* = 0.758)	−0.091 (*P* = 0.566)	−0.023 (*P* = 0.818)	0.131 (*P* = 0.186)	0.096 (*P* = 0.568)	0.309 (*P* = 0.002)	0.048 (*P* = 0.631)	−0.114 (*P* = 0.251)	–

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is 30–59.9 μg Se/l and severely deficient Se status is < 29.9 μg Se/l. In the above-mentioned research it was determined that the intragroup range of serum Se concentration in sheep varied enormously (45.4 ± 18.8 μg Se/l) as well as around 60% of the flocks showed serum Se concentrations below 80 μg/l, while 37.4% were below 60 μg Se/l, representing the Se deficient status. Although marginal deficiencies of trace elements are hard to detect, blood analyses may be an indicator of feeding problems in monitoring the animals, particularly for Se and Mo concentrations, before marginal deficiencies indicated clinical signs on animals which are visible (Haenlein and Anke 2011). As reported by Ademi et al. (2017) deficiencies of Se, Cu, Co, and Zn are all known as problems in different countries, thus information about these element levels in feed and animals is important for grazing animals. Sheep, as a grazing animal, are susceptible to nutritional deficiencies, because of local variation in the concentrations of these elements in pasture plants. The average Cu blood concentration of ewes in this study was in the adequate range which is 0.70–1.00 μg/dl according to Page et al. (2018). According to the NRC (2007) recommendation for Zn, Fe, Cu, Se, Mo and Co in feeds for sheep the range is 20–33 mg/kg, 30–50 mg/kg, 7–11 mg/kg, 0.1–0.2 mg/kg, 0.5 mg/kg and 0.10–0.20 mg/kg dry matter, respectively. Thereby, the green pasture in the present study had adequate amounts of Zn, Cu, higher Fe, Co and Mo, while Se concentration was lower through the experiment. Iron and Mo consumption was higher than the recommended level, but the Fe level below 500 mg/kg dry matter is considered as harmful (NRC 2007). For the blood serum Fe concentration Page et al. (2018) reported an adequate range of 116–122 μg/dl, marginal deficient concentration of 77–116 μg/dl, and deficient concentration as < 77 μg/dl. The determined Fe deficient concentration in the blood of ewes during lactation may be associated with more sources, for example with soil acidity as a low pH enhances Fe absorption by the plant. Page et al. (2018) reported an adequate range of 0.8–1.2 μg/ml for the blood serum Zn concentration, marginal deficient 0.6–0.8 μg/dl, and deficient Zn < 0.6 μg/ml. Considering the above information we did not determine any signs of deficiency in ewes during the experiment. Kulcu and Yur (2003) observed higher concentrations of K and Mg (25.61 mg/dl and 4.40 mg/dl) in the blood of Karakas sheep in lactation compared with our results. The serum Co concentrations in adult cattle are typically between 0.3 and 1.1 ng/ml (Herdt and Hof 2011), which is more compared to the present research. However, Page et al. (2018) reported adequate ranges of the blood serum concentration for Co > 0.10 ng/ml. In the present study low concentrations of Cd and As were determined. This can be related to the very low concentrations of Cd and As in the pasture which were mostly below the detection threshold (Table 1). Chemical and mainly metallurgical industry belongs to the main sources of air and environmental pollution with metals. The negative impact of the emission is visible in all branches of agricultural production (Broucek et al. 2009). Kovacik et al. (2017) determined higher concentrations of Cd (1.46 mg/kg), Zn (5.77 mg/kg) and Cu (0.98 mg/kg) in sheep blood in comparison with the present study. Environmental pollution and anthropogenic activity have a significant influence in an increased content of heavy metals in the environment. These changes indicated the preservation of the farm environment.

In ewes' blood a significant increase of Mg, Co and Cd concentrations was found with lactation, as well as a significant decrease of Na, Fe, Cu, Zn, Mo and Se concentrations. The lowest concentration of Ca in blood was found in the early stage, compared to the other stages. The drop in Ca concentrations during the early stage of lactation as the lactation progresses was determined by Antunovic et al. (2017b). Abdelrahman and Aljumaah (2012) also found lower concentrations of Ca in the blood of ewes during lactation. The reason could be the increased excretion of Ca through milk due to the increased milk production during early lactation. Abd-El Naser et al. (2014) determined significant changes in Ca concentration in the blood of cows during different stages of lactation while changes in P and Mg concentrations were not under the influence of lactation stage. The same authors found a significant increase in Ca concentrations from early to late lactation (from 3–6 weeks to 22–38 weeks), as well as a slight increase in Mg concentrations. In the present study the fluctuations of Ca were in positive correlation with K, Co and Cd, and in negative correlation with Cu and Mo. In the blood of Buchi sheep during lactation Kulcu and Yur (2003) found significantly lower concentrations of Cu and higher Mg, as well as insignificantly lower Zn and Mn in comparison with ewes before

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pregnancy and during pregnancy. These results were observed in the present study as Mg was increasing, and Cu was decreasing during lactation (r = –0.471). A similar negative correlation was determined between Mg and Se (r = –0.202), since Mg concentration was increasing and Se concentration was decreasing during lactation. Fluctuations of Se and Cu in ewes' blood resulted in positive correlations during lactation (r = 0.432). Changes in the concentration of electrolytes in the blood of ewes, particularly during lactation, were mainly associated with increased requirements for increased synthesis of milk in lactation (Kaneko et al. 2008). Sodium concentration dropped in ewes during lactation. Similar results in ewes were observed by Sobiech et al. (2008). Hu and Murphy (2004) observed that the serum levels of Na and K electrolytes in ruminants are independent of their concentrations in the diet. The serum levels of Na and K are determined primarily by their excretion through the kidneys. Significantly decreased Fe concentration in the blood of ewes in lactation could be due to increased excretion through milk and it could also be related to Fe content in pastures (Table 1). Similar fluctuations were observed in Cu concentration, which was confirmed by a positive correlation between Fe and Cu (r = 0.395). Concentration of Cu was positively correlated with Zn (r = 0.301), as was also determined in the research by El Zubeir et al. (2005) in the blood of cows during lactation (Cu : Zn, r = 0.68) as well as Kovacik et al. (2017) in the blood of ewes (Cu : Zn, r = 0.54).

Obtained correlations may help in monitoring the effect of nutrition on mineral concentration in blood providing information about the health status of ewes during the lactation period indicating if possible supplementation is needed. The detection of minerals in ewes' blood during lactation may present an indication of feeding problems or clinical signs if these concentrations are marginally deficient. Besides, minerals detection in the blood of ewes kept on pasture may be an indicator of mineral concentrations in soil and plants as well as their bioavailability in the area of Bilogora in Croatia.

CONCLUSION

In the present study significant changes in most macrominerals and trace elements in the blood of ewes were determined during lactation which were mostly within reference values. With lactation progress, in the blood of ewes significantly increased Ca, Mg, Co and Cd concentrations were found, as well as a significant decrease of Na and trace elements like Fe, Cu, Zn, Mo and Se concentrations. A significant number of correlations between the studied parameters indicated their association with ewes' metabolism. Obtained results may help in the prevention of various metabolic disorders in grazing ewes as a consequence of inadequate concentrations of macrominerals and trace elements in blood, usually derived from their inadequate concentrations in soil and pasture. Low concentrations of toxic elements in blood, like Cd and As, indicated well-preserved environment. To obtain a clearer idea related to the supply of macrominerals and trace elements to sheep in the future, an experimental design should include the analysis of soils from pastures as well as daily pasture consumption.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Abd-El Naser EM, Mohamed GAE, Elsayed HK. Effect of lactation stages on some blood serum biochemical parameters and milk composition in dairy cows. Assiut Vet Med J. 2014 Aug;60(142):83-8.

Abdelrahman MM, Aljumaah RS. Metabolic blood profiles and milk compositions of peri-parturient and early lactation periods in sheep. Asian J Anim Vet Adv. 2012 Nov;7 (11):1130-8.

Ademi A, Bernhoft A, Govasmark E, Bytyqi H, Sivertsen T, Singh BR. Selenium and other mineral concentrations in feed and sheep's blood in Kosovo. Transl Anim Sci. 2017 Feb;1(1):97-107.

Antunovic Z, Novoselec J, Klir Z. Hematological parameters in ewes during lactation in organic farming. Poljoprivreda. 2017a Nov;23(2):46-52.

https://doi.org/10.17221/250/2020-CJAS

Antunovic Z, Novoselec J, Speranda M, Steiner Z, Cavar S, Pavlovic N, Valek Lendic K, Mioc B, Pacinovski N, Klir Z. Monitoring of blood metabolic profile and milk quality of ewes during lactation in organic farming. Mljekarstvo. 2017b Sep;67(4):243-52.

Broucek J, Soch M, Srejberova P. Effect of different environmental factors on selected blood minerals in sheep. Slovak J Anim Sci. 2009 Sep 30;42(2):145-50.

EU 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. OJEU. 2010 Oct 20;L276: 47 p.

El Zubeir EMI, Elowni OAO, Mohamed GE. Correlation of minerals and enzymes in blood serum and milk of healthy and mastitic cows. Res J Agric Biol Sci. 2005 Jan;1(1):45-9.

Haenlein GFW, Anke M. Mineral and trace element research in goats. A review. Small Rumin Res. 2011 Jan 1; 95(1):2-19.

Herdt TH, Hoff B. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Vet Clin North Am Food Anim Pract. 2011 Jul 1;27(2):255-83.

Hu W, Murphy MR. Dietary cation-anion difference effects on performance and acid-base status of lactating dairy cows: A meta-analysis. J Dairy Sci. 2004 Jul 1;87(7):2222-9.

Humann-Ziehank E, Tegtmeyer PC, Seelig B, Roehrig P, Ganter M. Variation of serum selenium concentrations in German sheep flocks and implications for herd health management consultancy. Acta Vet Scand. 2013 Nov 19; 55(1): [8 p.].

Jukna C, Jukna V, Suigzdaite J. Determination of heavy metals in viscera and muscles of cattle. Bulg J Vet Med. 2006 Jan;9(1):35-41.

Kaneko JJ, Harvey JW, Bruss ML. Clinical biochemistry of domestic animals. 6th ed. London: Elsevier/Academic Press; 2008. 931 p.

Kovacik A, Arvay J, Tusimova E, Harangozo L, Tvrda E, Zbynovska K, Cupka P, Andrascikova S, Tomas J, Massanyi P. Seasonal variations in the blood concentration of selected heavy metals in sheep and their effects on the biochemical and hematological parameters. Chemosphere. 2017 Feb 1;168:365-71.

Kulcu R, Yur F. A study of some serum mineral levels before and during pregnancy and during lactation period of sheep and cattle. Biol Trace Elem Res. 2003 Jun;92(3): 275-80.

Lopez-Alonso M. Trace minerals and livestock: Not too much not too little. ISRN Vet Sci. 2012 Dec;2012: [18 p.].

Novoselec J, Gregurincic I, Klir Z, Mioc B, Siric I, Drzaic V, Antunovic Z. The estimation of body weight from body measurements of Travnik Pramenka sheep in the area of Bilogora, Croatia. J Cent Eur Agr. 2020 Jun;21(2):207-14.

NRC – National Research Council. Nutrient requirements of small ruminants. Washington, D.C.: The National Academy Press; 2007. 347 p.

Page CM, Murphy TW, Van Emon ML, Bowman JGP, Wyffels SA, Stewart WC. Blood serum mineral element concentrations of weaned Montana ram lambs and their relationship with water quality characteristics. Prof Anim Sci. 2018 Oct 1;34(5):410-20.

Popenga RH, Ramsey J, Gonzales BJ, Johnson CK. Reference intervals for mineral concentrations in whole blood and serum of bighorn sheep (Ovis canadensis) in California. J Vet Diagn Invest. 2012 May;24(3):531-8.

Puls R. Mineral levels in animal health: Diagnostic data. 2nd ed. Clearbrook (BC): Sherpa International; 1994. p. 302-53.

Ramirez-Perez AH, Buntix SE, Rosiles R. Effect of breed and age on the voluntary intake and the micromineral status of non-pregnant sheep. II. Micromineral status. Small Rumin Res. 2000 Aug 1;37(3):231-42.

Schweinzer V, Iwersen M, Drillich M, Wittek M, Tichy A, Mueller A, Krametter-Froetscher R. Macromineral and trace elements supply in sheep and goats in Austria. Vet Med-Czech. 2017 Feb 13;62(2):62-73.

Sobiech P, Milewski S, Zdunczyk S. Yield and composition of milk and blood biochemical components of ewes nursing a single lamb or twins. Bull Vet Inst Pulawy. 2008 Aug;52(4):591-6.

Suttle NF. Mineral nutrition of livestock. 4th ed. Wallingford: CABI Publishing; 2010. 587 p.

Underwood EJ, Suttle NF. The mineral nutrition of livestock. 3rd ed. Wallingford: CABI Publishing; 1999. 614 p.

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