Selenium and other mineral concentrations in feed and sheep's blood in Kosovo[1]

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ABSTRACT: The aim of this study was to assess the concentration of Se and other minerals in sheep and the supplied feed. Four macrominerals (Ca, P, Mg, and S), 7 microminerals (Se, Fe, Zn, Cu, Mn, Co, and Mo), and 2 toxic minerals (Cd and Pb) were analyzed in 69 feed and 292 sheep blood samples from 30 farms in different regions of Kosovo. The samples were analyzed using inductively coupled plasma mass spectrometry, and mineral concentrations in whole blood were measured to assess their status in animals. Concentrations of the different minerals in feed were found in the following ranges: 1.9 to 9.5 g Ca/kg DM, 0.8 to 3.2 g P/kg DM, 0.8 to 3.2 g Mg/kg DM, 1.0 to 2.8 g S/kg DM, 6 to 82 μg Se/kg DM, 33 to 970 mg Fe/kg DM, 15 to 42 mg Zn/kg DM, 2.6 to 7.5 mg Cu/kg DM, 26 to 250 mg Mn/kg DM, 0.04 to 0.88 mg Co/kg DM, 0.05 to 0.86 mg Mo/kg DM, 0.07 to 2.02 mg Pb/kg DM, and 0.02 to 0.19 mg Cd/kg DM. Concentrations of the microminerals analyzed in whole blood were found in the following ranges: 15 to 360 μg Se/L, 190 to 500 mg Fe/L, 1.4 to 3.8 mg Zn/L, 0.3 to 2.6 mg Cu/L, 6 to 243 μg Mn/L, 0.1 to 19.6 μg Co/L, and 1.8 to 66.0 μg Pb/L. Among all minerals, the largest deficiency was found for Se both in feed and sheep blood, with 82% of feed samples and 83% blood samples being inadequate, and its supplementation is necessary. Selenium-supplemented sheep had significantly higher Se concentration in blood than non-supplemented sheep ($P < 0.01$). In addition, other macro- and microminerals in feed such as P, S, Cu, and Co were at inadequate concentrations at some of the farms, and supplementation may also be needed for these minerals.

Key words: animal feed, Kosovo, lambs and ewes, mineral deficiency, whole blood

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INTRODUCTION

Minerals have different specific functions in the animal body, such as structural, physiological, catalytic, and regulatory, although many of the minerals have multiple functions (Suttle, 2010; McDonald et al., 2011). Sheep's requirements for different minerals are widely described in literature. However, there is not always agreement among researchers regarding these requirements, because different factors are taken into consideration for requirement calculations. There are extra requirements for growth (weight gain), milk or wool production, and animal reproduction within the same animal species (McDowell, 2003; NRC, 2007; Suttle, 2010). Data from NRC (1985, 2007) are frequently used as a reference for sheep and goats requirements for minerals worldwide. However, mineral requirements data of NRC are based on US centric data, and mineral availability, and to a certain level requirements may differ in countries outside the US. Sheep are grazing animals, getting most of their nutrients from pasture and locally grown roughage. They are therefore vulnerable to nutritional deficiencies, especially of essential minerals, because of local variation in the levels of these elements in grass and pasture plants. For instance, deficiencies of Se, Cu, Co, and Zn are all known as

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clinical problems in different countries. For sheep farming, information about these element levels in feed and animals are therefore important. In Kosovo, cases of Se deficiency in sheep were previously reported (Ademi et al., 2015). Also, a low concentration of Se in soil (lower than 500 μg/kg) and subsequently in plants (lower than 50 μg/kg), was found in another survey study conducted by our group (unpublished data). However, data concerning essential minerals in feed and in animal blood are rather scant in Kosovo. Therefore, the main objective of the present study was to examine the status of minerals in sheep and their available feeds at different farms of Kosovo. Additional objectives were to evaluate the relationship between minerals in sheep's blood and their feed, the effect of supplementation, and regional variation on the mineral status of sheep.

MATERIAL AND METHODS

This research study was done in accordance with both national regulation of Norway and Kosovo for the use of animals under study: Norwegian Animal Welfare Act (LOV-2009-06-19-97), Norwegian Regulation on Animal Experimentation (FOR-1996-01-15-23), and Kosovo Law on Animal welfare (Law No.02/L-10). Qualified veterinarians have performed blood collection.

Study Area and Sample Collection

The experimental study consisted of 30 farms in 6 different districts of Kosovo, Western Balkans. At each individual farm, blood was sampled from 5 ewes and 5 lambs. However, 3 of the farms did not have lambs. Ewes older than 2 yr and lambs from 1 to 6 mo were selected for the purpose of sampling. Blood sample from each animal was taken by jugular venipuncture into Vacuette trace mineral tubes with sodium heparin (Greiner Bio-One/VWR international, Kremsmunster, Austria) and stored cold during the sampling period. These samples were later frozen at –18°C until the chemical analysis was performed. In addition, feed samples comprising 34 samples of hay, 29 samples of cereal grain, and 5 samples of silage were collected from the same farms. The number of feed samples from each farm was either 2 or 3 depending on the feed used by the farmer. Feed samples were dried at 105°C and then finely ground before oxidative digestion. The finely ground feed samples and blood samples were digested as described below.

Sample Digestion and Chemical Analysis

One milliliter of whole blood was digested in an Ultra Clave (Milestone Inc., Sheltone, CT) with 5 mL of concentrated nitric acid. The procedure for feed digestion was the same but the amount of feed sample digested was 250 mg with 2 mL of ultrapure water and 5 mL of nitric acid. In each sample, 250 μL of a solution containing 4 mg/L indium, thallium, tellurium, and rhodium was added as internal standard before digestion. After digestion, samples were diluted to 50 mL with ultrapure water and left for stabilization at least overnight before mineral measurements were done. Total mineral concentration in all digested samples of blood and feed was determined using inductively coupled plasma mass spectrometry (Agilent Technologies 8800 series, Santa Clara, CA), and oxygen was used as a reaction gas in the collision cell.

For the quality control of the analytical method, different certified reference materials, such as Wheat Flour 1567a, Bovine Serum 1598, and Dogfish Liver DOLT4, were digested for each set of digestions, and the total mineral concentration was determined. Certified reference materials concentrations were within certified ranges. Three blanks were run for each digestion set to correct the measurements. The limit of detection (LOD) was calculated as 3 × SD of the blanks and limit of quantification (LOQ) was calculated as 10 × SD of the blanks. Concentration of all minerals presented was above LOQ, except for a few samples of Co and Pb. For statistical calculations, sample concentrations between LOD and LOQ were set to (LOD + LOQ) / 2, whereas samples below LOD were not considered for statistical analyses.

Collection of Information from Farmers

A questionnaire containing information about type and amount of feed (percentage of different feed types used at the farm) and type of mineral supplementation (if any) was prepared and provided to each farm before sampling (Supporting Document 1). The questionnaires with the desired information were collected during sampling visits. Based on this information, animals (farms) were grouped into mineral-supplemented and non-supplemented animals (farms) for statistical analyses. In addition, supplemented animals were grouped according to the type of supplementation.

Data Processing and Statistical Analysis

Feed samples were analyzed for 4 macrominerals (Ca, P, Mg, and S), 7 microminerals (Se, Fe, Zn, Cu, Mn, Co, and Mo), and 2 toxic minerals (Cd and Pb). For practical purposes, feed samples were grouped in 3 categories: grain (from various cereals), hay (from grass, alfalfa, or green wheat), and silage (from maize or grass). Because samples were collected on extensive farms where indoor feeding during winter is based mainly on

roughages, the information from questionnaires on the percentage of cereal grains used in the total feed ration on each farm was only approximate. Based on this information, we estimated that 80% of the total feed used was roughage (hay and silage, and if both were available for use at the farm, we presumed equal usage, i.e., 40% of each type) and 20% was cereal grains. Based on these ratios, we calculated an estimated mineral concentration of the total feed ration at each farm, using formula below:

$$\begin{aligned} &\textit{Mineral conc in available feed} \\ &= \frac{40}{100} \times \textit{mineral conc in grass} + \frac{40}{100} \times \textit{mineral conc in silage} \\ &+ \frac{20}{100} \times \textit{mineral conc in concentrated feed} \end{aligned}$$

Whole blood samples of sheep were analyzed for all minerals mentioned above. However, because whole blood concentration of the macrominerals generally does not reflect the nutrient intake of these minerals, we did not include this data in the manuscript.

The R statistical program, version 3.0.1, R Commander, version 2.0–4, was used for data processing. An ANOVA model was used to test the significant effect of different factors such as animal age group (ewes and lambs), feed type, mineral supplementation, farm, and region. Tukey's honest significant difference at a level of significance of 0.05 was used to test the difference in means among these factors. Pearson product correlation was used to test the correlation between the mineral concentration in blood and the feed consume at the time. Only winter-collected samples have been considered for this relationship, as all feed types were analyzed during this period. For summer-collected blood samples, it was impossible to compare them with respect to mineral concentration intake from pastures, because farmers frequently move their sheep from one place to another.

RESULTS AND DISCUSSIONS

Mineral Concentration in Feed

Mean concentrations and ranges of different macrominerals in the total feed rations at the farms, as well as mean concentrations of these minerals in different feed types and sheep requirements, are given in Table 1.

The 2 major elements Ca and P are frequently described together, because of the specific interaction between them. For adequate intake of these minerals, not only their concentration in the feed is important but also an adequate Ca:P ratio. In this study, the mean concentration of Ca in the total feed was 5.0 g/kg DM and ranged from 1.9 to 9.5 g/kg DM among farms (Table 1), which is mostly within the range of 2.0 to 8.2 g/kg DM reported for sheep requirements (NRC, 1985, 2007; McDowell, 2003). Calcium was below the minimum requirement only at 1 of the farms. Based on that, Ca seems to be adequate for maintenance and production, but it is inadequate for pregnant sheep with multiple fetuses on the last weeks of pregnancy as well as on the onset of lactation, when a greater concentration of Ca in the feed is required (up to 8.2 mg/kg of feed; NRC, 2007). As also reported in the literature (Suttle, 2010), hay and silage were shown to be significantly better sources of Ca than grain (mean concentrations of 6.0, 4.8, and 0.6 g/kg DM, respectively; Table 1). Phosphorus ranged from 0.8 to 3.2 g/kg DM with a mean concentration of 2.0 g/kg DM (Table 1). Based on the requirements from 1.6 to 3.8 g/kg DM for sheep (NRC, 1985, 2007; McDowell, 2003), P in feed was inadequate at least 6 farms and above the maximum requirement at 6 farms. The inadequate concentration of P found at some of the farms from our study is not surprising, because P deficiency is reported as the most prevalent mineral deficiency for grazing ruminants worldwide (McDowell, 2003; Suttle, 2010). Unlike Ca, P was significantly higher in grain than in hay and silage (mean concentrations of 3.8, 1.7, and 1.5 g/kg DM, respectively; Table 1), and such a difference was also previously reported (Suttle, 2010). A Ca:P ratio between 1 and 2.4:1 is considered ideal for normal growth and bone formation (McDowell, 2003; NRC, 2007). In the present study, the ratio varied between 0.8 and 8.6:1, and at 14 of the 30 farms, it was higher than the required upper limit. From the results, it is obvious that P deficiency and a high Ca:P ratio are of more concern than Ca deficiency in these areas. Phosphorus deficiency per se is responsible for reduction in feed intake, lameness, weight loss, and reduced reproduction (Jubb and Crough, 1988; Shupe et al., 1988; Ternouth and Sevilla, 1990; Dunn and Moss, 1992). Although clinical signs of P deficiency were not an objective of this study, symptoms such as lameness, weight loss, and low reproduction are frequent in the region.

The mean concentration of Mg in the total feed was 1.7 g/kg DM (SD 0.5), ranging from 0.8 to 3.2 g/kg DM (Table 1). Sheep requirements for Mg were reported to range from 1.2 to 1.8 g/kg DM (NRC, 1985; McDowell, 2003). Hence, Mg concentration in the feed was lower than the minimum requirement at 3 farms. However, Mg concentration as low as 0.7 mg/kg DM is considered adequate for maintenance in the most recent data published by the NRC (2007). No significant variation in Mg concentration between hay and cereal grains was shown in the present study, which is in accordance with other reports (McDowell, 2003; Suttle, 2010). It is known that the risk of acute hypomagnesemia (also known as grass tetany) is highest in grazing animals in the spring and autumn

Table 1. Mean mineral concentrations in animal feed and differences between feed types, along with requirements and MTL[1]

			No. of farms							
	Total diet (30)		Lower than minimum requirement	Lower than maximum requirement	Greater than MTL	Type of feed				
						Grain (29)	Hay (34)	Silage (5)		
Mineral	Mean (SD)	Ranges				Mean (SD)			Requirement[2]	MTL
Ca, g/kg DM	5.0 (2.0)	1.9–9.5	1	26	0	0.6 (0.8)[a]	6.0 (2.7)[b]	4.8 (3.1)[b]	2.0–8.2	15
P, g/kg DM	2.0 (0.6)	0.8–3.2	6	24	0	3.8 (1.4)[a]	1.7 (0.6)[b]	1.5 (0.7)[b]	1.6–3.8	6
Ca:P ratio	2.7:1	0.8–8.6:1	2	16	1	0.15:1	3.7:1	5.5:1	1:1–2.4:1	7:1
Mg, g/kg DM	1.7 (0.5)	0.8–3.2	3	18	0	1.5 (0.6)[a]	1.8 (0.8)[a]	1.8 (0.6)[a]	1.2–1.8	6
S, g/kg DM	1.7 (0.5)	1.0–2.8	7	29	0	1.4 (0.2)[a]	1.9 (0.7)[b]	1.0 (0.4)[a]	1.4–2.6	3 (5)[3]
Se, mg/kg DM	27 (18)	6–82	30	0	0	30 (25)[a]	24 (17)[b]	31 (29)[a]	100–200	5000
Fe, mg/kg DM	190 (218)	33–970	0	1	2	69 (13)[a]	200 (270)[a]	88 (41)[a]	30–50	500
Zn, mg/kg DM	24 (6)	15–42	6	28	0	33 (13)[a]	22 (7)[b]	28 (12)[a,b]	20–33	300
Cu, mg/kg DM	5.1 (1.2)	2.6–7.5	28	2	0	4.1 (2.4)[a]	5.2 (1.8)[a]	5.6 (3.2)[a]	7–11	15[4]
Mn, mg/kg DM	81 (60)	26–250	0	9	0	31 (28)[a]	96 (70)[b]	35 (12)[a]	20–40	2000
Co, mg/kg DM	0.21 (0.22)	0.04–0.88	14	22	0	0.03 (0.06)[a]	0.27 (0.23)[b]	0.06 (0.02)[a,b]	0.1–0.2	25
Mo, mg/kg DM	0.36 (0.23)	0.05–0.86	NA[5]	19	0	0.37 (0.28)[a]	0.36 (0.28)[a]	0.26 (0.16)[a]	> 0.5*	5
Pb, mg/kg DM	0.34 (0.37)	0.07–2.02	NA	NA	0	0.08 (0.16)[a]	0.38 (0.44)[b]	0.20 (0.15)[b]	NA	100
Cd, mg/kg DM	0.07 (0.04)	0.02–0.19	NA	NA	0	0.02 (0.02)[a]	0.1 (0.06)[b]	0.06 (0.02)[a,b]	NA	10 (1)[6]

[a,b]Values with different superscripts significantly differ ($P < 0.05$).

[1]MTL = maximum tolerable levels (based on data published by the NRC (2005).

[2]Requirements for minerals in diet are based on data published by McDowell (2003) and the NRC (1985).

[3]Sulfur MTL are given for both the high-concentrate diet and the high-forage diet (the last one in parentheses).

[4]MLT for Cu is given assuming normal concentrations of Mo (1 to 2 mg/kg DM diet) and S (0.15 to 0.25%). At Mo and S concentrations below these, Cu may become toxic at lower levels.

[5]NA = Not applicable.

[6]Cadmium MTL in parentheses represents the upper limit in complete feed for animals used for human consumption set by the World Health Organization (World Health Organization/International Programme on Chemical Safety WHO/IPCS, 1992).

(McDowell, 2003), although hypomagnesemia can also happen in indoor-fed animals (winter tetany). According to our results on feed Mg, clinical hypomagnesemia is less likely to happen in animals fed indoors, because the feed ration at most of the farms contained sufficient amounts of Mg for the animals' maintenance.

The mean concentration of S in the feed was 1.7 g/kg DM (SD 0.5), ranging from 1.0 to 2.8 g/kg DM (Table 1). Sheep's requirement for S is reported to range from 1.4 to 2.6 g/kg DM (NRC, 1985, 2007; McDowell, 2003); hence, in our study, feed S was below the minimum requirement at 7 of the farms, indicating a possibility of feed S deficiency for sheep. As reported by others (McDowell, 2003; Suttle, 2010), and also found in our study, hay contained slightly higher S concentration than grain and silage (mean concentrations of 1.9, 1.4, and 1.0 g/kg DM, respectively; Table 1). Sulfur deficiency in ruminants is characterized by symptoms such as loss of appetite, reduced weight gain, and reduced wool production (Qi et al., 1992, 1994; McDowell, 2003; Suttle, 2010). For non-ruminants, total S content in the feed is not as important as the presence of S-containing amino acids, whereas the opposite is true for ruminants (McDowell, 2003; Suttle, 2010). Ruminal bacteria are able to create S-containing amino acids for their own and host animal needs, provided there is a proper ratio of S to N in the feed (Kandylis, 1984; Henry and Ammerman, 1995; McDowell, 2003; Suttle, 2010).

The mean concentration of Se in the total feed at different farms was 27 μg/kg DM, ranging from 6 to 82 μg/kg DM (Table 1). Grain and silage were slightly but significantly higher in Se concentration than hay (mean concentrations of 30, 31, and 24 μg/kg DM, respectively; Table 1), although high variation was observed among the same types of feed. The reported requirement for Se in feed is 100 to 200 μg/kg DM, and feed with Se content lower than 50 μg/kg DM is considered deficient (NRC, 1985, 2007; McDowell, 2003). At all the investigated farms feed Se was inadequate, but at 25 farms (83%) it was found to be clearly deficient (below 50 μg/kg DM). Similarly, low concentrations of Se in the feed were previously reported from other Western Balkan countries, such as Serbia (Maksimovic et al., 1992), Croatia (Antunovic et al., 2010), and Bosnia and Herzegovina (Muratovic et al., 2007).

The mean concentration of Fe in the feed was 190 mg/kg DM (SD 218), with high variation among farms (ranging from 33 to 970 mg/kg DM; Table 1). Compared with data from literature (NRC, 1985, 2007;

McDowell, 2003), Fe in feed was found to be higher than the requirement (30 to 50 mg/kg DM) at all sampled farms, and at 2 of them, it was even higher than the maximum tolerable level (MTL; 500 mg/kg DM; Table 1). Similarly, high values for Fe have been previously reported from the Western Balkans, possible because of contamination from soil (Manojlovic and Singh, 2012). Excessive amounts of Fe, found in most of the samples, are reported to reduce uptake of other elements such as Cu, P, Zn, and Mn (Standish et al., 1971; Fontenot et al., 1989; McDowell, 2003; Suttle, 2010).

Zinc in the feed ranged from 15 to 42 mg/kg DM, with a mean concentration of 24 mg/kg DM (SD 6; Table 1). Compared with the sheep requirement of 20 to 33 mg/kg DM of feed (NRC, 1985, 2007; McDowell, 2003), Zn was found to be inadequate at 6 farms. Zinc deficiency is widespread around the world and it is not only the matter of Zn deficiency in soil but also of its bioavailability to plants and animals (Cakmak et al., 1999; Alloway, 2008; Cakmak, 2008).

The mean concentration of Cu in the feed was 5.1 mg/kg DM (SD 1.2) and ranged from 2.6 to 7.5 mg/kg DM (Table 1). According to reported sheep requirements (7 to 11 mg/kg DM; NRC, 1985, 2007; McDowell, 2003), Cu in the feed samples from this study was within the requirement limits only at 2 of the investigated farms and it was inadequate at 28 of the farms (Table 1). This suggests that Cu is inadequate in the feed and supplementation may be needed. However, there are other issues to consider before deciding for supplementation because of the narrow difference between its MTL and the maximum requirement (15 and 11 mg/kg DM, respectively). In addition, the contents of Mo and S (known as Cu antagonists) in the feed in our study were generally at or below the lower limit of requirement (Table 1). The MTL of 15 mg/kg DM was set assuming the normal concentrations of Mo (1 to 2 mg/kg DM diet) and S (0.15 to 0.25%), with a warning that at the lower content of these 2 antagonists, Cu may become toxic at lower concentrations (NRC, 2007). Thus, in total the Cu concentration in feed for sheep in Kosovo may be at a relatively sufficient level.

Manganese in the total feed was found to vary markedly from farm to farm, ranging between 26 and 250 mg/kg DM (Table 1). The feed at all farms was found to contain Mn within or above the requirement range for sheep (20 to 40 mg/kg DM; NRC, 1985, 2007; McDowell, 2003). In addition, Mn varied markedly between feed types. Hay was found to have significantly higher Mn concentrations than both grain and silage (mean concentrations 96, 31, and 35 mg/kg DM, respectively; Table 1), which is in accordance with other results reported in the literature (McDowell, 2003; Suttle, 2010). Contamination from soil was reported as a possible reason for the higher concentration of Mn in hay (Suttle, 2010). At measured concentrations of Mn in the feed, no need for Mn supplementation is suggested.

Cobalt in the total feed varied markedly between farms, ranging from 0.04 to 0.88 mg/kg DM (Table 1). Based on the requirement of Co for sheep from 0.1 to 0.2 mg/kg DM of feed (NRC, 1985, 2007; McDowell, 2003), it was found to be inadequate at least 14 of the investigated farms. Cobalt in hay was significantly higher than in cereal grains (mean concentrations of 0.27 and 0.03 mg/kg DM, respectively; Table 1). Because Co was deficient in several of the feed samples and ruminants, unlike non-ruminants, need Co for synthesis of cobalamin (vitamin B_{12}) by ruminal bacteria, Co supplementation must be considered in the area. Some researchers enlisted subclinical Co deficiency as one of the major causes of poor production and economic losses in sheep (Latteur, 1962; McDowell, 2003), and it has often gone unnoticed.

Like Co, Mo in total feed was found to have a wide range, from 0.05 to 0.86 mg/kg DM, with a mean concentration of 0.36 mg/kg DM (SD 0.23; Table 1). Although the minimum requirement for Mo is not known, a concentration of Mo higher than 0.5 mg/kg DM is stated as required for sheep (NRC, 1985, 2007; Suttle, 2010). At 19 farms, Mo was below this concentration and possibly inadequate for optimal growth and production.

Lead and Cd, considered toxic elements, were below the MTL reported: 100 mg Pb/kg DM and 1 mg Cd/kg DM (World Health Organization/International Programme on Chemical Safety (WHO/IPCS), 1992a,b; NRC, 2005). Lead ranged from 0.07 to 2.02 mg/kg DM of feed and it was significantly lower in grain than in hay and silage (mean concentrations of 0.08, 0.38, and 0.20 mg/kg DM, respectively; Table 1). As for Fe and Mn, higher concentration of Pb in hay and silage may be caused by soil contamination. Cadmium was found to range from 0.02 to 0.19 mg/kg DM, and it was lower in cereal grains than in hay and silage (mean concentrations of 0.02, 0.1, and 0.06 mg/kg DM of feed, respectively; Table 1).

Mineral Concentrations in Sheep Whole Blood

For several of the minerals, concentrations in animal plasma and serum are more commonly reported, rather than minerals concentrations in whole blood (Kincaid, 1999; Herdt and Hoff, 2011). There are only few studies that reported minerals concentrations in whole blood of animals and humans (Georgievskii et al., 1982; Harrington et al., 2014). On the other hand, whole-blood Se concentration is more frequently analyzed, because it expresses the long-term Se status in animals, whereas the plasma or serum concentration may fluctuate within a

Table 2. Mean trace element concentrations in sheep whole blood and difference between ewes and lambs, along with reference values

	All sheep			Ewes		Lambs			Reference values[2]			
									Animals		Humans	
Minerals	No.	Mean (SD)	Ranges	No.	Mean (SD)	No.	Mean (SD)	IA,[1]%	Whole blood	Serum/plasma	Whole blood	Serum/plasma
Se, µg/L	292	81 (58)	15–360	150	85 (59)	132	76 (57)	82	120–350	60–200	58–234	46–143
Fe, mg/L	158	360 (60)	190–500	74	350 (50)[a**3]	60	380 (60)[b]	48	360–420	0.9–2.7	309–521	0.75–1.5
Zn, mg/L	292	2.4 (0.5)	1.4–3.8	150	2.3 (0.5)[a*]	132	2.5 (0.4)[b]	100	2.5–6.0 (4–5)	0.55–1.2	4.4–8.6	0.7–1.2
Cu, mg/L	292	0.8 (0.2)	0.3–2.6	150	0.80 (0.20)	132	0.82 (0.25)	53	0.8–1.2	0.75–1.7	0.8–1.3	0.8–1.7
Mn, µg/L	292	25 (19)	6–243	150	25 (21)	132	26 (16)	98	70–200	1.0–6.0	8.0–18.7	0.54–1.76
Co, µg/L	292	1.9 (3.1)	0.1–19.6	150	1.4 (2.2)[a**]	132	2.5 (3.8)[b]	5	(30–50)	(5–10)	0.24–0.82	0.11–0.45
Pb, µg/L	292	9 (9)	1.8–66.0	150	7 (6)[a***]	132	13 (10)[b]	NA[3]	–	–	8–269	< 1.0

[a,b]Values with different superscripts significantly differ.

[1]IA = inadequate animals; animals with inadequate mineral concentration in whole blood.

[2]Reference values are based on the literature (Georgievskii et al., 1982; Iyengar and Woittez, 1988; McDowell, 2003; Herdt and Hoff, 2011; Kincaid, 1999; Harrington et al., 2014).

[3]NA = Not applicable.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3. Correlation coefficient (r) of minerals in whole blood with respective or other minerals in feed

		Minerals in feed												
Minerals		Se	Fe	Zn	Cu	Mn	Co	Pb	Cd	Mo	Ca	P	Mg	S
Se	Blood	0.67***[1]	0.32***	0.05	–0.02	–0.35***	0.14	0.03	–0.27**	0.16	–0.04	0.21*	0.21*	0.12
	Feed	1	0.10	0.10	0.06	–0.25**	–0.04	–0.13	–0.14	0.27**	–0.12	0.24**	0.28**	0.01
Fe	Blood	–0.01	0.19*	–0.04	–0.12	–0.03	0.12	0.17	–0.13	–0.11	–0.01	–0.05	0.01	–0.07
	Feed	0.10	1	–0.15	–0.15	–0.34***	0.77***	0.93***	–0.38***	–0.04	–0.11	–0.15	0.07	–0.15
Zn	Blood	0.23*	0.14	0.07	–0.04	0.01	0.01	0.04	–0.05	–0.21*	–0.04	–0.03	0.04	0.19
	Feed	0.10	–0.15	1	0.77***	0.50***	–0.10	–0.14	0.58***	–0.29***	0.53***	0.02	0.47***	0.71***
Cu	Blood	0.26**	–0.09	0.16*	0.08	0.06	–0.13	–0.18	0.03	–0.39*	–0.05	0.12	–0.11	–0.06
	Feed	0.06	–0.15	0.77***	1	–0.00	0.03	–0.18*	0.33***	–0.10	0.79***	0.49***	0.65***	0.64***
Mn	Blood	0.16	–0.12	–0.03	–0.10	–0.00	–0.11	–0.16	–0.14	0.00	–0.05	0.05	0.05	0.12
	Feed	–0.25**	–0.34***	0.50***	–0.00	1	–0.49***	–0.14	0.73***	–0.55***	–0.12	–0.70***	–0.14	0.16
Co	Blood	0.00	0.52***	–0.19*	–0.16	–0.20*	0.42***	0.53***	–0.24**	–0.03	–0.12	–0.11	0.03	–0.24**
	Feed	–0.04	0.77***	–0.10	0.03	–0.49***	1	0.77***	–0.52***	0.11	0.23**	0.24**	0.14	–0.02
Pb	Blood	–0.05	0.32***	–0.07	–0.09	–0.11	0.30***	0.34***	–0.25**	–0.14	0.01	0.02	0.03	–0.06
	Feed	–0.13	0.93***	–0.14	–0.18*	–0.14	0.77***	1	–0.31***	–0.17*	–0.10	–0.29***	0.02	–0.22*

[1]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 4. Effect of type and time of supplementation on mineral concentration in whole blood

Mineral supplement[1]	No.	Minerals in whole blood [mean (SD)]						
		Se, µg/L	Fe, mg/L	Zn, mg/L	Cu, mg/L	Mn, µg/L	Co, µg/L	Pb, µg/L
No supplement	83	40 (17)^{a}	344 (58)^{a}	2.3 (0.5)^{a,b,c}	0.76 (0.21)^{a}	25 (12)	1.2 (0.9)^{a}	9.4 (8.7)^{a,b}
Injectable selenium	40	130 (92)^{c}	401 (60)^{b}	2.6 (0.4)^{b}	0.92 (0.33)^{b}	30 (22)	1.3 (2.2)^{a,b}	11.6 (10.5)^{b}
Earlier	20	52 (14)	390 (72)	2.4 (0.3)	0.91 (0.45)	25 (25)	0.6 (0.3)	6.1 (3.5)
Recently	20	209 (65)	406 (56)	2.7 (0.4)	0.94 (0.15)	34 (18)	2.0 (2.9)	17.0 (12.4)
Mineral blocks	33	80 (21)^{b}	NA[2]	2.1 (0.4)^{a}	0.74 (0.17)^{a}	22 (9)	3.5 (4.2)^{c}	5.2 (3.3)^{a}
Earlier	23	78 (22)	NA	2.1 (0.4)	0.74 (0.19)	24 (10)	4.9 (4.4)	6.0 (3.7)
Recently	10	86 (21)	NA	2.1 (0.3)	0.76 (0.10)	18 (7)	0.3 (0.1)	3.5 (1.2)
Mineral premixes	116	96 (54)^{b}	367 (53)^{a,b}	2.4 (0.4)^{b,c}	0.82 (0.19)^{a,b}	26 (24)	2.4 (3.9)^{b,c}	9.4 (8.7)^{a,b}
Earlier	79	72 (23)	364 (60)	2.3 (0.5)	0.79 (0.17)	27 (28)	2.2 (4.0)	6.8 (5.3)
Recently	37	147 (64)	369 (49)	2.6 (0.3)	0.88 (0.23)	23 (10)	2.7 (3.6)	14.8 (11.6)
Feed compound	20	73 (34)^{b}	351 (36)^{a}	2.3 (0.4)^{a,b}	0.75 (0.12)^{a}	24 (11)	1.3 (2.1)^{a,b,c}	9.9 (7.7)^{a,b}
Just started	10	43 (7)	346 (35)	2.4 (0.4)	0.78 (0.12)	21 (4)	0.8 (0.0)	6.8 (2.8)
Recently	10	104 (19)	355 (37)	2.2 (0.3)	0.72 (0.12)	27 (14)	1.8 (2.9)	13.0 (9.7)
Total mean	292	81 (58)	362 (56)	2.4 (0.5)	0.80 (0.22)	25 (19)	1.9 (3.1)	9.0 (9)

$^{a–c}$Values with different superscripts significantly differ ($P < 0.05$).

[1]"No supplement" indicates no use of any mineral supplements, "Earlier" indicates use of mineral supplements before at least 2 mo, "Recently" indicates use of mineral supplements during last 2 mo, and "Just started" indicates use of mineral supplements for less than a month.

[2]NA = Not applicable.

shorter period of time and is therefore more an indicator of recent Se intake from the daily feed (Suttle, 2010). Because the main focus in the present study was on Se status of animals, we decided to analyze whole blood for both Se and other minerals reported.

Selenium ranged from 15 to 360 µg/L (Table 2), with 82% of samples being inadequate according to reference values (120–350 µg/L) reported by Herdt and Hoff (2011). A whole-blood Se concentration as low as 100 µg/L is considered adequate by other researchers (Van Saun et al., 1989; Gerloff, 1992; Fordyce, 2005). However, nearly 78% of animals in the present study were found to have Se concentrations below this limit as well. Similar blood Se concentration was found in lambs and ewes. Blood Se correlated very well ($r = 0.67$) with Se concentration in the feed, and significant positive correlation ($r = 0.32$) with feed Fe was also observed (Table 3). In addition, significantly positive correlation of blood Se with feed Mg and P and negative correlation with Mn and Cd in the feed seems to reflect the good correlations between these minerals in the feed (Table 3). Although correlation of Se in blood and feed is frequently reported (Govasmark et al., 2005; Hall et al., 2013), correlations of Se in blood with Fe, Mg, Mn, and Cd in the feed have not been previously reported. Naturally, the strongest factor influencing blood Se concentration in sheep was found to be Se supplementation. All types of Se supplementations increased Se concentration in sheep blood (mean concentration 130 µg Se/L for injectable Se, 80 µg Se/Lfor mineral blocks, 96 µg Se/L for mineral premixes, and 73 µg Se/L for compound feed) compared with non-supplemented sheep (41 µg Se/L; Table 4), with highest concentrations of Se in sheep supplemented through injectable Se (with vitamin E). In addition, both supplementation time before sampling and its duration influenced Se concentration in the blood. Sheep injected with Se more than 2 mo before sampling showed significantly lower blood Se than those injected more recently [less than 1 mo before sampling; 52 (SD 14) and 209 µg/L (SD 65), respectively], and in the former case, sheep showed Se concentrations close to the non-supplemented ones (Table 4). This is reasonable, as it has been previously reported that Se concentration in liver and kidneys can drop to a baseline as soon as 23 d after Se injection (Van Vleet, 1975). Similarly, sheep supplemented by mineral premixes or compound feed during the last 2 mo showed significantly higher blood Se than sheep supplemented more than 2 mo ago or those that just started supplementation (Table 4).

The only significant difference concerning blood Se among regions was between Gjakova and Ferizaj (mean concentrations 144 and 60 µg/L, respectively; Table 5), with sheep farms from Gjakova having higher Se concentrations, most probably because of elevated use of Se supplementation.

All over the world, farm animals are susceptible to Se deficiency, and nutritional muscular dystrophy or white muscle disease (WMD) is frequent, with calves and lambs being most susceptible (Whanger, 1989; McDowell, 2003; Fordyce, 2005). Selenium deficiency is also accompanied by slower weight gain, reduced fertility, immune depression, and an increased incidence of intramammary infections, and in severe deficiency, it leads to heart muscle degeneration and sudden death (Droke and Loerch, 1989; Hogan et al., 1990; Suttle, 2010). Although no clinical signs of Se deficiency were

Table 5. Whole blood mineral concentration in different regions

Regions	No.	Minerals						
		Se, µg/L	Fe, mg/L	Zn, mg/L	Cu, mg/L	Mn, µg/L	Co, µg/L	Pb, µg/L
Ferizaj	64	60 (25)^{c}	363 (53)	2.2 (0.5)^{b}	0.79 (0.17)^{b,c}	21 (10)	0.8 (0.8)^{c}	9.5 (9.8)^{a,b}
Gjakove	32	144 (75)^{a}	357 (39)	2.6 (0.4)^{a}	0.96 (0.20)^{a}	29 (11)	4.9 (5.6)^{a}	8.4 (4.3)^{a,b}
Gjilan	55	95 (71)^{b}	364 (68)	2.4 (0.5)^{a,b}	0.84 (0.31)^{a,b}	26 (14)	0.7 (0.5)^{c}	9.5 (7.8)^{a,b}
Peje	44	72 (73)^{b,c}	350 (60)	2.4 (0.4)^{a,b}	0.78 (0.17)^{b,c}	29 (21)	1.6 (2.1)^{b,c}	8.3 (9.0)^{a,b}
Prishtine	54	72 (35)^{b,c}	365 (51)	2.3 (0.4)^{b}	0.68 (0.15)^{c}	24 (32)	2.1 (3.0)^{b,c}	12.0 (10.5)^{a}
Prizren	43	71 (26)^{b,c}	366 (65)	2.4 (0.5)^{a,b}	0.84 (0.23)^{a,b}	28 (11)	3.2 (3.7)^{b}	6.5 (5.7)^{b}
Total	292	81 (58)	362 (56)	2.4 (0.5)	0.80 (0.22)	25 (19)	1.9 (3.1)	9.2 (8.6)

$^{a–c}$Values with different superscripts significantly differ ($P < 0.05$).

observed during sampling at the farms, even with such low Se concentrations in feed and blood, most farmers at the farms included in the present study knew about or had been faced with WMD at their own farm in the past. Therefore, Se supplementation must be considered absolutely necessary to avoid the risk of WMD and subtle disorders due to Se deficiency.

In this study, Fe ranged between 190 and 500 mg/L in whole blood, which is slightly lower than reference values previously reported for sheep and human whole blood (360 to 420 and 309 to 521 mg/L, respectively (Georgievskii et al., 1982; Iyengar and Woittiez, 1988; Harrington et al., 2014). The mean Fe concentration was at the lower limit of reference values for sheep, and 48% of the samples were below the limit (Table 2). In general, Fe-deficiency anemia in ruminants is not related with Fe content in the feed but is mainly a result of hemorrhages caused by intestinal parasites (Kolb, 1963; Abbott et al., 1988; McDowell, 2003). High content of Fe in the feed from the sampled farms and rather low but significant correlation between Fe content in sheep and in their feed ($r = 0.19$, Table 3) supports the possibility of intestinal parasitosis in sampled sheep with low content of Fe, although control for parasites was not an objective of this study. Iron in blood was significantly higher in sheep injected with Se than in non-supplemented sheep and those fed by compound feed in addition to hay (mean concentrations 401, 344, and 351 mg/L, respectively; Table 4). An increased sensitivity to hemolysis in Se-deficient rats has been reported (Rotruck et al., 1972; Kim et al., 1988). In addition, an anemia associated with the presence of Heinz bodies and suboptimal blood Se, which was corrected after supplementation with Se, was reported in grazing cattle in Florida (Morris et al., 1984; McDowell and Williams, 1991). Therefore, the higher concentration of Fe in Se-injected sheep may possibly be related to a protective effect of Se in the membrane of erythrocytes and reduced hemolysis. Iron was significantly higher in lambs than in ewes (mean concentrations of 380 and 350 mg/L, respectively; Table 2). To our knowledge, such difference between ewes and lambs has not been previously reported and could possibly be a result of differences in the number of erythrocytes in blood.

Zinc concentrations in whole blood ranged between 1.4 and 3.8 mg/L (Table 2), which is lower than the reference values reported for sheep and human whole blood (4.0 to 5.0 and 4.4 to 8.6 mg/kg DM, respectively; Georgievskii et al., 1982; Harrington et al., 2014). Because all of the blood samples in our study were below the lower reference limit for sheep whole blood and the mean concentration in feed samples was close to the lower limit of adequacy, there is a strong indication of inadequate Zn status in the area. There was no significant correlation between whole-blood Zn and feed Zn (Table 3). The only significant correlation was between blood Zn and feed Se ($r = 0.23$). It seems that mineral supplementation through mineral blocks, mineral premixes, or compound feed is not adequate as sufficient Zn supplementation, and maybe more available forms of Zn or Zn-biofortified feed should be considered for supplementation. The Zn concentration in lambs was slightly but significantly higher than in ewes. The Zn concentration in sheep was slightly higher in the region of Gjakova than in other regions but significantly different only from the regions of Ferizaj and Prishtina (Table 5).

The mean Cu concentration was 0.8 mg/L, with a range from 0.3 to 2.6 mg/L (Table 2). About 53% of all blood samples had Cu concentrations lower than the reference ranges for both sheep and human whole blood (0.8 to 1.2 and 0.8 to 1.3 mg/kg DM, respectively; Georgievskiĭ et al., 1981; Harrington et al., 2014), and only 1 sample had a Cu concentration higher than the upper reference limit reported. Although Cu was close to the lower limit in both sheep blood and their feed, no significant correlation between them was observed. However, it has been reported that the blood concentration of Cu is maintained relatively stable from liver stores and does not drop unless severe deficiencies in feed for a long period of time are observed (McDowell, 2003; Suttle, 2010). Negative correlations of liver Cu with Mo and S content in feed has been reported (McDowell, 2003). In this study, there was a significant negative correlation of blood Cu with Mo in feed ($r = –0.39$), but

correlation with S or other minerals in feed was not significant, possibly because of high correlation between Cu and S in the feed ($r = 0.64$, Table 3). Sheep injected with Se had the highest concentration of Cu, and their concentrations were significantly different from those of non-supplemented sheep as well as from those of sheep supplemented by mineral blocks or compound feed (Table 4). Similar blood Cu concentration was found in lambs and ewes. Only a slight variation between regions was observed, and the only significant difference was between Gjakova and Prishtina (mean concentrations 0.96 and 0.68 mg/L, respectively; Table 5).

Manganese in blood was found to vary from 6 to 243 μg/L (Table 2), and it was similar to previous findings reported for sheep or human whole blood (Georgievskii et al., 1982; Harrington et al., 2014). However, according to other researchers (Hidiroglou, 1979, 1980; Kincaid, 1999), adequate concentration of Mn in cattle whole blood is from 70 to 200 μg/L. If the same ranges could be applied for sheep, then only 2% of sheep samples could be considered adequate in Mn. About 37% of the samples were found to be below 20 μg/L, considered the limit of deficiency in cattle blood (Hidiroglou, 1979; Kincaid, 1999). There was no significant correlation between blood Mn and Mn or any other minerals in feed (Table 3), and type of supplementation had no effect on blood Mn (Table 4). Although Mn was found in a relatively wide range, no difference between lambs and ewes or regional variations was observed (Table 5). Considering that Mn concentration in feed was within or higher than requirements for sheep (NRC, 2007; Table 1), it is possible that the adequate concentration for sheep whole blood may be lower than those reported for cattle.

Cobalt in our study ranged between 0.1 and 19.6 μg/L (Table 1). To our knowledge, there are no comprehensive data regarding adequate blood concentration of Co in animals or humans, because blood concentration of vitamin B_{12} is usually measured and reported instead of blood Co. Hence, it is a bit difficult to state any definite adequate level of Co for whole blood. However, Harrington et al. (2014) reported Co in human's whole blood to be between 0.24 and 0.82 μg/L, and if we can assume a similar concentration for sheep, then 56% of the samples are higher than reference values reported. According to Georgievskii et al. (1982), whole blood Co in ruminants is from 30 to 50 μg/L, which would imply that all of our samples are possibly deficient. Cobalt was slightly but significantly higher in lambs than in ewes, although high variation within both lambs and ewes were observed (Table 2). Blood Co correlated well with its content in feed. Also, it correlated well with Fe and Pb in feed, but this positive correlation may reflect the high correlation of these 3 minerals in feed (Table 3). Sheep supplied with mineral blocks had a significantly higher concentration of Co in blood (Table 4). Cobalt concentration in sheep was significantly higher in the region of Gjakova than in all other regions (Table 5).

Lead, a toxic element, ranged from 1.8 to 66.0 μg/L, which is lower than mean background blood concentrations given for sheep (0.09 mg/L) and cattle (0.10 mg/L) given by Osweiler et al. (1985) and lower than or within the ranges reported for human blood (Harrington et al., 2014; Table 2). Lead concentration in blood was slightly but significantly higher in lambs than in ewes (mean concentrations 13 and 7 μg/L, respectively; Table 2). Blood Pb was significantly correlated with Pb and Fe content in feed, consistent with their good correlation in the feed (Table 3).

Molybdenum, both an essential and a toxic element, and Cd, a toxic element, were also measured in blood samples. Both were found to be below the detection limit in most of the samples and are hence not presented.

Conclusions

Among the 4 macrominerals analyzed, P and S showed deficiency levels in the feed. This implies a need for supplementation of these 2 minerals to provide maximum performance and health. Although Ca per se was generally within the requirements for sheep, it might be inadequate for sheep in last weeks of pregnancy and the onset of lactation. On the other hand, the high Ca:P ratio found in this study may indicate lower availability of P. Because different concentration of Ca and P were found in hay/silage and cereal grains, with Ca being higher in hay/silage and P being higher in cereal grains, adequate ratios of these feed types should be provided to avoid their deficiencies. Sulfur is insufficient, particularly for wool producing and growing sheep. Hence, its supplementation is almost necessary for these categories of sheep.

Among the microminerals Se was the most deficient in both feed and blood samples, and supplementation is particularly important for Se. All types of supplementation registered in the study increased Se concentration in blood, but the greatest effect was observed in sheep injected with Se. Copper levels in the feed were also below recommended requirements, and the moderate Cu concentrations in blood could imply a possible need for Cu supplementation. However, low feed concentrations of Mo and S, both being antagonists to Cu, and the narrow difference between tolerance and requirement for Cu in sheep indicate that care should be taken not to introduce a toxicity risk. Although Zn in feed was inadequate only at some farms, its low concentration in whole blood is an indication that supplementation

might be needed. However, mineral supplementation had no effect on whole blood Zn concentration; hence, a bioavailable form of Zn must be used or Zn-biofortified feed should be considered for supplementation.

Cobalt, being at the lowest level of sheep requirements in feed, should possibly also be supplemented. Cobalt had higher concentrations in sheep supplemented by mineral blocks and mineral premixes than in non-supplemented sheep. Manganese was at an adequate concentration in feed, but based on reference levels for cattle it was possibly at an inadequate concentration in blood, and should maybe be supplemented.

Based on the conclusions given above we would recommend farmers to consider sheep supplementation with Se and Zn. That seems particularly important in the region of Ferizaj, where we found the lowest levels of these minerals in the animals. Because there was no correlation between blood and feed Zn, use of Zn biofortified feed must be considered. Supplementation of Co and Mn might also be beneficial.

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