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Macromineral and trace element supply in sheep and goats in Austria

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ABSTRACT: The aim of this study was to determine the supply of 25 different macrominerals (calcium, magnesium, potassium) and trace elements (aluminium, arsenic, barium, boron, cadmium, cobalt, copper, iron, lithium, lead, manganese, molybdenum, nickel, selenium, silicon, strontium, sulphur, thallium, tin, titanium, uranium, zinc), and to ascertain the presence of any over- or undersupplies. As a second objective, we undertook a comparison of our results with existing reference values from selected literature and from laboratory analyses, with the aim of classifying the obtained results into the following categories: 'deficiency', 'adequate' and 'oversupply'. For the study, 16 sheep and four goat farms located in the Austrian states of Upper Austria (n = 12), Carinthia (n = 6) and Vorarlberg (n = 2) were selected. From every farm, five serum blood samples were obtained by puncturing the *vena jugularis* to evaluate the macromineral and trace element status in clinically sound female sheep (n = 80; 12 different breeds) and female goats (n = 20; Saanen goats, Boer goats). The animals were kept for dairy farming (milking and/or meat production) or for landscaping. The mean age of both sheep and goats was 3.1 years (sheep: min. 0.5, max. 10; goats: min. 1, max. 5); 44% of the studied animals were lactating and 22% were pregnant at the time of sampling. The serum blood samples were sent to a laboratory and analysed using inductively coupled plasma optical emission spectrometry and inductively coupled plasma mass spectrometry. In summary, the supply with macrominerals and trace elements compared with reference values from the laboratory was adequate for As, Ca, Fe and Mg in sheep and for As, Ca, Cu, K, Mg and Se in goats. Although all animals in our study were examined for clinical signs of disease by the local veterinarian, oversupplies in sheep for the elements K and Mo and in goats for Fe as well as undersupplies in sheep and goats for Zn could be found in the serum of the studied animals.

Keywords: small ruminant; minerals; element status; deficiency; oversupply; serum; reference value; ICP-MS; IPC-OES

Some macrominerals and trace elements are classified as essential mineral elements. This classification is based on the concentration needed in the diet and in animal tissues (Herdt and Hoff 2011), and does not reflect the importance of the mineral (Rankins and Pugh 2012).

Certain macrominerals are required in large quantities and include calcium, chloride, magnesium, phosphorous, potassium, sodium and sulphur. Essential microminerals or trace elements include cobalt, copper, iodine, iron, manganese, molybdenum, selenium and zinc. These elements are required in smaller quantities (Fraga 2005).

For humans, animals and plants, these minerals and trace elements are essential for all biological processes. They are part of structural components of tissues, act as electrolytes in fluids, and serve as catalysts in the endocrine system or in enzymes (McDowell 2003). Macrominerals and trace elements are required for all physiological functions, and support optimal growth, health condition, the immune system, productivity and reproduc-

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tion (Hostetler et al. 2003; Herdt and Hoff 2011; Pavlata et al. 2011; Poppenga et al. 2012).

An inadequate supply of macrominerals and trace elements will lead to a deficiency and cause biochemical dysfunction, disturbed physiological functions or structural disorders (McDowell 2003). For example, macrominerals such as K are important for osmotic pressure and normal acid-base balance. Mg is involved in enzymatic reactions and is required for normal operation of the nervous system, and Ca is mostly found in skeletal tissues and is essential for muscle contraction (Sykes 2007; Rankins and Pugh 2012). Some trace elements are particularly important for ruminants, for example for rumen digestion (Co, Cu, Mo), performance (Co, Cu, Fe, I, Mn, Mo, Se, Zn), fertility (Co, Cu, I, Mn, Se, Zn) and for fitness (Cu, Fe, Mn, Se; Moritz 2014).

Rankins and Pugh (2012) described that a deficiency of trace elements is less frequent than a deficiency in proteins or energy supply, whereas Herdt and Hoff (2011) stated that nutritional deficiencies or metabolic disturbances in trace elements are relatively common. Compromised immune status is one important consequence of low levels of trace elements. Trace element deficiency and infectious diseases are often found together, because trace elements play an important role in the defence against infectious organisms (Bhaskaram 2002; Or et al. 2005; Fouda et al. 2011).

Signs of deficiency may develop over long periods of time, in a manner which depends on the mineral, the extent and time of exposure or deficiency, as well as on individual factors, e.g. species, age, sex, pregnancy, lactation and whether clinical signs are obvious (Radostits et al. 2007; Herdt and Hoff 2011). Undersupply of elements can also occur when soil or plants contain low levels of the mineral, the elements interfere with the utilisation of the plant or if certain elements interfere with each other, resulting in reduced resorption and a secondary deficiency (Hefnawy and Tortora-Perez 2010; Haenlein and Anke 2011). Undersupply in grazing ruminants is a frequent phenomenon (Humann-Ziehank et al. 2008), because most grazing sheep and goats do not receive mineral supplements at all, or if these are administered only on an *ad libitum* basis. Furthermore, dietary concentrations are often unknown and vary depending on season, geographical region and forage species (McDowell 2003; Khan et al. 2007). In contrast to cows, routine analyses of blood samples are less commonly performed in small ruminants and there is a lack of information in the literature regarding the prevalence of deficiency or oversupply. The prevailing attitude, especially in dairy sheep and dairy goats, is that these ruminants can be treated as small cows and that, therefore, research data from dairy cows can simply be extrapolated down to these animals. However, extensive mineral literature can be found in the published annual meeting proceedings of the International Goat Association (1982, 1987, 1992, 1996, 2000, 2004), national annual meetings in France (ITOVIC), Mexico (UANL), England (BDSA) and in the published annual proceedings of the Mineral and Trace Element Symposia at the University of Jena, Germany, since 1975. This information, however, is often not available for practitioners in the field.

To determine the status of trace element supply, it is possible to analyse blood samples, organs (kidney, liver, muscles or brain), hair or milk. For determining macrominerals the most common technique is to analyse blood samples. These methods will be discussed later in the manuscript. The objective of this study was to evaluate the macromineral and trace element status of clinically sound sheep and goats in Austria. First, we set out to determine the general macromineral and trace element supply status in these animals, and further, to ascertain whether there was any deficiency or oversupply in the study population. A second objective was to compare our results with reference values from the literature, available to most practitioners. It was not our aim in this study to define new reference values, but rather to discuss our results within the context of existing references and to provide new insights into the status of macromineral and trace element supply in Austria.

MATERIAL AND METHODS

Study design, animals, feeding and housing. The study period was from April to May 2014. Sixteen sheep and four goat farms located in different regions of Austria were selected for inclusion. All farms either kept only sheep or goats, never both. Eleven of the sheep farms and one goat farm were located in Upper Austria, five sheep farms and one goat farm were situated in Carinthia and two goat farms were in Vorarlberg. All sheep and goats included in the study were females. Twelve differ-

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ent sheep breeds (Bergschaf, Cameroon sheep, East Friesian, Ile-de-France, Jura, Karntner Brillenschaf, Lacaune, Merino sheep, Schwarzkopf, Shropshire sheep, Steinschaf and Texel sheep) and two goat breeds (Boer goats and Saanen goat) were included in the study. The mean age was 3.1 years for sheep (min. 0.5, max. 10) and 3.1 years for goats (min. 1, max. 5). In total, 36% (27/75) of the sheep and 75% (15/20) of the goats were lactating and 28% (21/75) of the sheep and none of the goats were pregnant. The animals were kept for dairy farming, meat production or for landscaping. In the winter months all sheep and goats from two farms were housed in a stable and were at pasture during the summer months. The goats of the remaining two farms were kept in a stable throughout the entire year.

All sheep and goat farms fed normal hay and additionally, nine sheep farms and two goat farms fed grass silage, and one sheep farm fed grass and corn silage. Furthermore, six sheep and three goat farms supplied their animals with concentrates. Nine sheep and three goats farms fed mineral blends and 14 sheep and three goat farms provided licking stones; therefore, minerals were supplemented through feeding for most of the animals. None of the animals were fully grazing throughout the months of April and May.

Blood sampling. On every farm, blood samples from five sheep or five goats were collected by the local veterinarian in the context of routine herd health management and diagnostics. The farms were selected as a convenience sample based on the personal contact of the veterinarian to the owner. On every farm blood samples were taken from five apparently healthy, adult animals, which were selected by the veterinarian to avoid bias by the owner.

In total, 100 blood samples were collected, with 80 from sheep and 20 from goats. Before blood sampling, all animals were examined for clinical signs of disease by the local veterinarian. Only clinically sound animals were sampled and included in this study. Blood was obtained by puncturing the *vena jugularis* and was collected in sterile vacutainer tubes (Vacuette®, Greiner Bio One GmbH, Frickenhausen, Germany) coated with micronized silica particles as clot activator. After collection, tubes were immediately cooled. The blood was centrifuged on the day of collection, and serum was removed and kept frozen at –20 °C pending analysis. The concentrations of macrominerals and trace elements in serum were analysed by Idexx Vet Med Labor GmbH, Ludwigsburg, Germany. A total of 95 serum samples were analysed (75 sheep and 20 goat serum blood samples); five samples could not be analysed because of haemolysis. The samples were analysed using inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian Vista Pro®, Varian Medical Systems, Inc., California, USA) and inductively coupled plasma mass spectrometry (ICP-MS) (Varian 820 MS®, Varian Medical Systems, Inc., California, USA). Both techniques are used to measure the concentration of macrominerals and trace elements. The detection limit for ICP-MS is lower than that for ICP-OES. The following elements were measured using ICP-MS: arsenic, cadmium, cobalt, lead, lithium, nickel, selenium, thallium, tin and uranium. ICP-OES was used to analyse the following elements: aluminium, barium, boron, calcium, copper, iron, magnesium, manganese, molybdenum, potassium, silicon, strontium, sulphur, titanium and zinc.

Statistical analyses. All data were collected and coded for statistical analyses in Microsoft Excel (version 6.1.760, Microsoft Cooperation, Redmond, USA). The supply with different minerals is presented as boxplots. Standard descriptive statistics (mean ± SD, minimum, maximum) were performed with SPSS Statistics for Windows (version 20.0; IBM Deutschland GmbH, Ehningen, Germany).

Reference values for macrominerals and trace elements from the laboratory were compared with selected reference values. As references, three textbooks (Radostits et al. 2007; Baumgartner 2009; Pugh and Baird 2012) and one review (Herdt and Hoff 2011) were chosen. These references were selected because they are available and common for practitioners in Austria and around the world. The supply of macrominerals and trace elements was evaluated by classifying the results from the serum blood of sheep and goats into the following categories: 'deficiency', 'adequate', and 'oversupply'. Only elements with reference values available from the selected literature and the laboratory were evaluated and categorised. Elements without reference values from the selected literature and the laboratory were not categorised into groups, and were only displayed graphically.

RESULTS

In total, 95 serum blood samples of sheep (n = 75) and goats (n = 20) were analysed for their concen-

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trations of macrominerals and trace elements. In total, 25 different macrominerals (Ca, K, Mg) and trace elements (Al, As, B, Ba, Cd, Co, Cu, Fe, Li, Mn, Mo, Ni, Pb, S, Se, Si, Sn, Sr, Ti, Tl, U, Zn) were analysed. For 11 elements (As, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Se, Zn) reference values were available in the literature or were provided by laboratory analyses, but for nine elements (Al, Ba, B, Li, Ni, S, Si, Sr, Ti) no reference values were available. A further five elements (Cd, Pb, Sn, Tl, U) were not compared with reference values from the literature, because their levels were under the detection limit (less than 1 µg/l) of ICP-OES and ICP-MS.

Results for elements with available reference values in selected literature and from laboratory analyses for sheep and goats are presented as boxplots in Figures 1 and 2. Additionally, for some elements available laboratory reference values are added. For the trace elements Co and Mn a laboratory reference value was not available and, therefore, no reference lines were added. For As and Mo only, a reference line was added under which the supply is adequate. Boxplots of elements without reference values from selected literature and laboratory analyses are presented in Figures 3 and 4. Some results of sheep and goats were under the detection limit and therefore, they were excluded from being presented as boxplots in Figures 3 and 4.

The elements Al, As, Co, Cu, Mg, Ni and Ti showed a small range of values for sheep and goats, whereas B, Ba, Fe, Li and Zn in sheep and goats, and Mo and Se in sheep showed a large range. Ca and Mg in sheep and goats, Fe in sheep, and K and Se in goats, were elements that were within the laboratory reference values. In contrast, Cu and Zn in sheep and goats, K in sheep, and Fe in goats, were outside laboratory reference values. A large number of results were above the reference line from the laboratory for Mn. Furthermore, Ba, Li, Mn, Mo and S showed some outliers for sheep. The elements As, Co, Ni and Ti were found in very low concentrations compared to other elements. In contrast, Cu, S and Si were found at higher concentrations in the serum of sheep and goats.

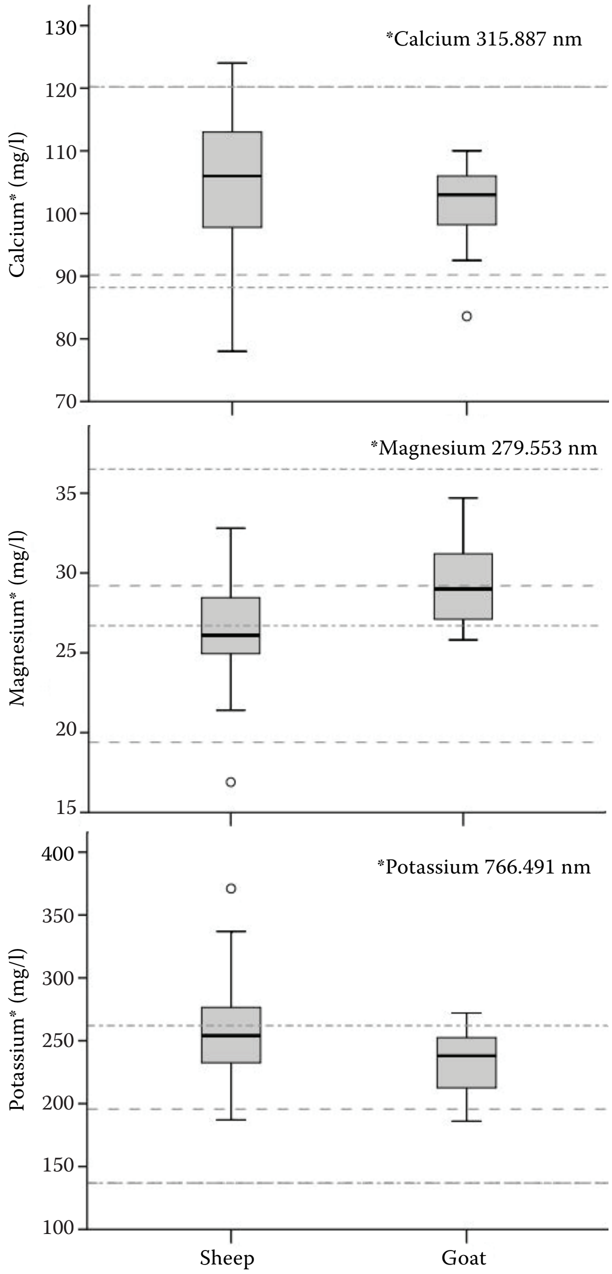

Figure 1. Boxplots of macrominerals including results of serum blood samples of 75 sheep and 20 goats; reference lines of the laboratory reference values (Idexx Vet Med Labor GmbH, Ludwigsburg, Germany) are added (dashed line = sheep, dot-dashed line = goats); black line in grey box = median, grey box = upper/lower quartile, whisker = minimum/maximum, ○ = outlier

Sheep

Available reference values from selected literature, the laboratory reference values and descriptive statistics of the test results of the sheep in the study population are provided in Table 1. The concentration of macrominerals and trace elements (As, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Se, Zn) in serum blood of the tested sheep were compared to values obtained for some minerals within the

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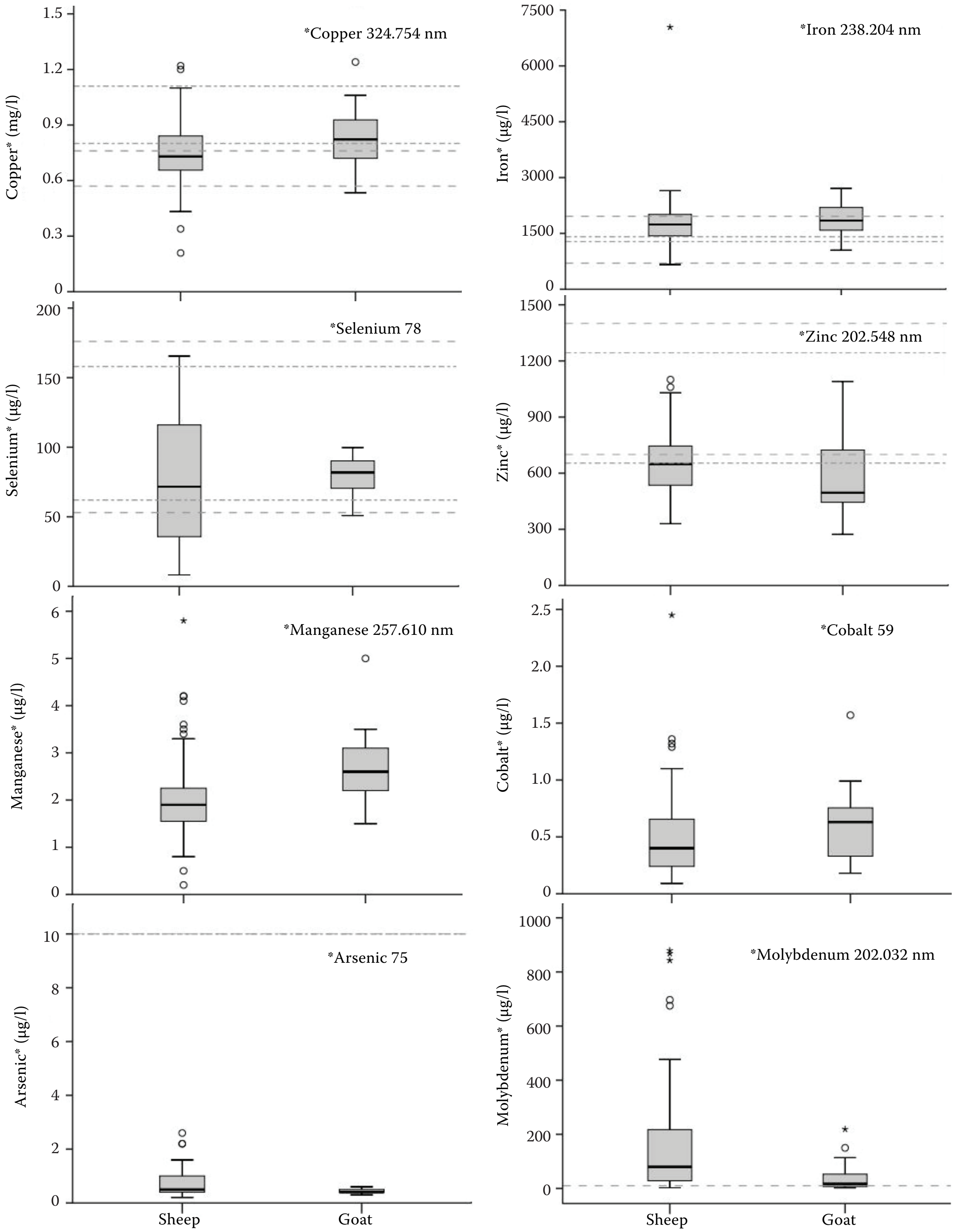

Figure 2. Boxplots of trace elements including results of serum blood samples of 75 sheep and 20 goats; reference lines of the laboratory reference values (Idexx Vet Med Labor GmbH, Ludwigsburg, Germany) are added (dashed line = sheep, dot-dashed line = goats); black line in grey box = median, grey box = upper/lower quartile, whisker = minimum/maximum, ○/★ = outlier

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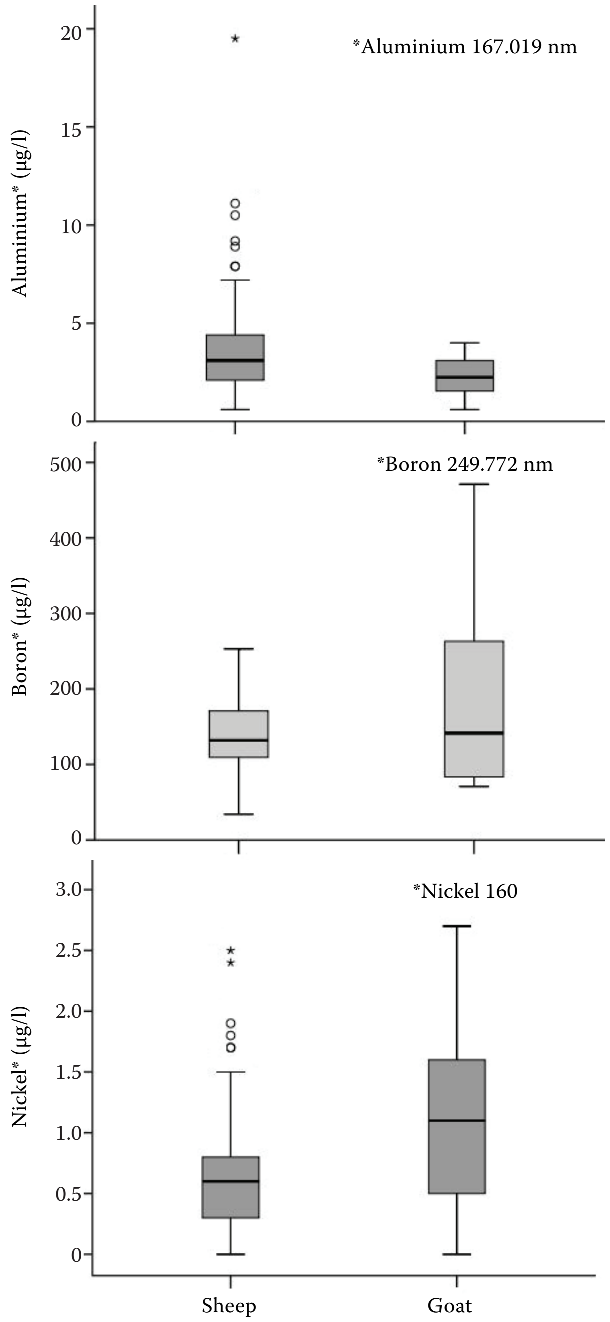

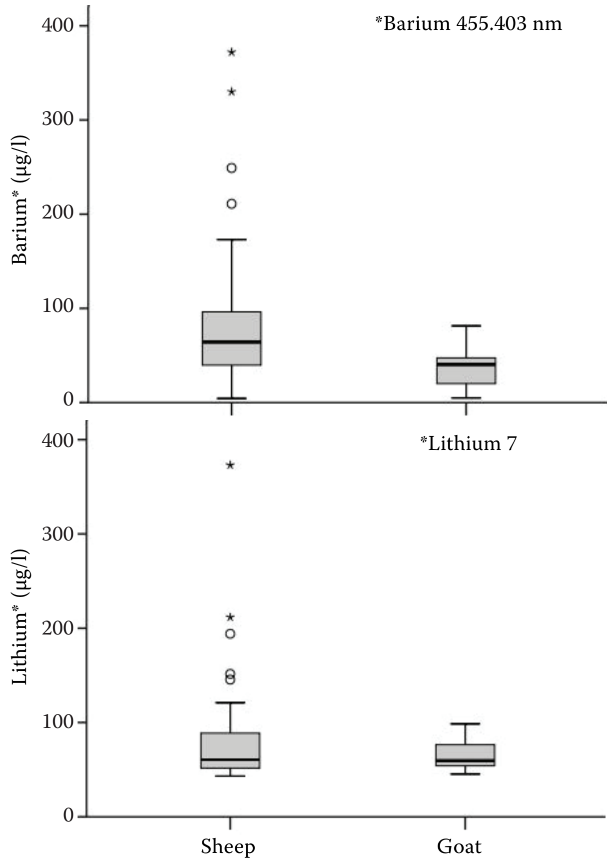

Figure 3. Boxplots of trace elements without reference values from selected literature and the laboratory (Idexx Vet Med Labor GmbH, Ludwigsburg, Germany) including results of serum blood samples of 75 sheep and 20 goats (except for Al (*n*= 74 sheep, *n* = 20 goats), Ni (*n* = 73 sheep, *n* = 18 goats)); black line in grey box = median, grey box = upper/lower quartile, whisker = minimum/maximum, ○/★ = outlier

reference range, while for others this analysis was not performed. Fewer reference values are available in the selected literature for the elements As, Co, Mn and Mo.

The supply of macrominerals and trace elements was evaluated based on the information in Table 1 and categorised into 'deficiency', 'adequate' and 'oversupply', according to the five different references. The results are summarised in Table 2. According to different references, over 85% of sheep in the study population had an oversupply of K and more than 69% were adequately supplied with Mg. Furthermore, references from the literature and the laboratory both indicated that Fe was adequately supplied. Additionally, the supply with Zn was three times classified as deficient and only once as adequate. A poor concordance was found between the information of the selected literature and the laboratory for the elements Ca, Cu and Se. For the trace elements As, Co, Mn and Mo reference values for sheep were rarely available in the selected literature (Table 1). Whereas for Co, Mn and Mo two differ-

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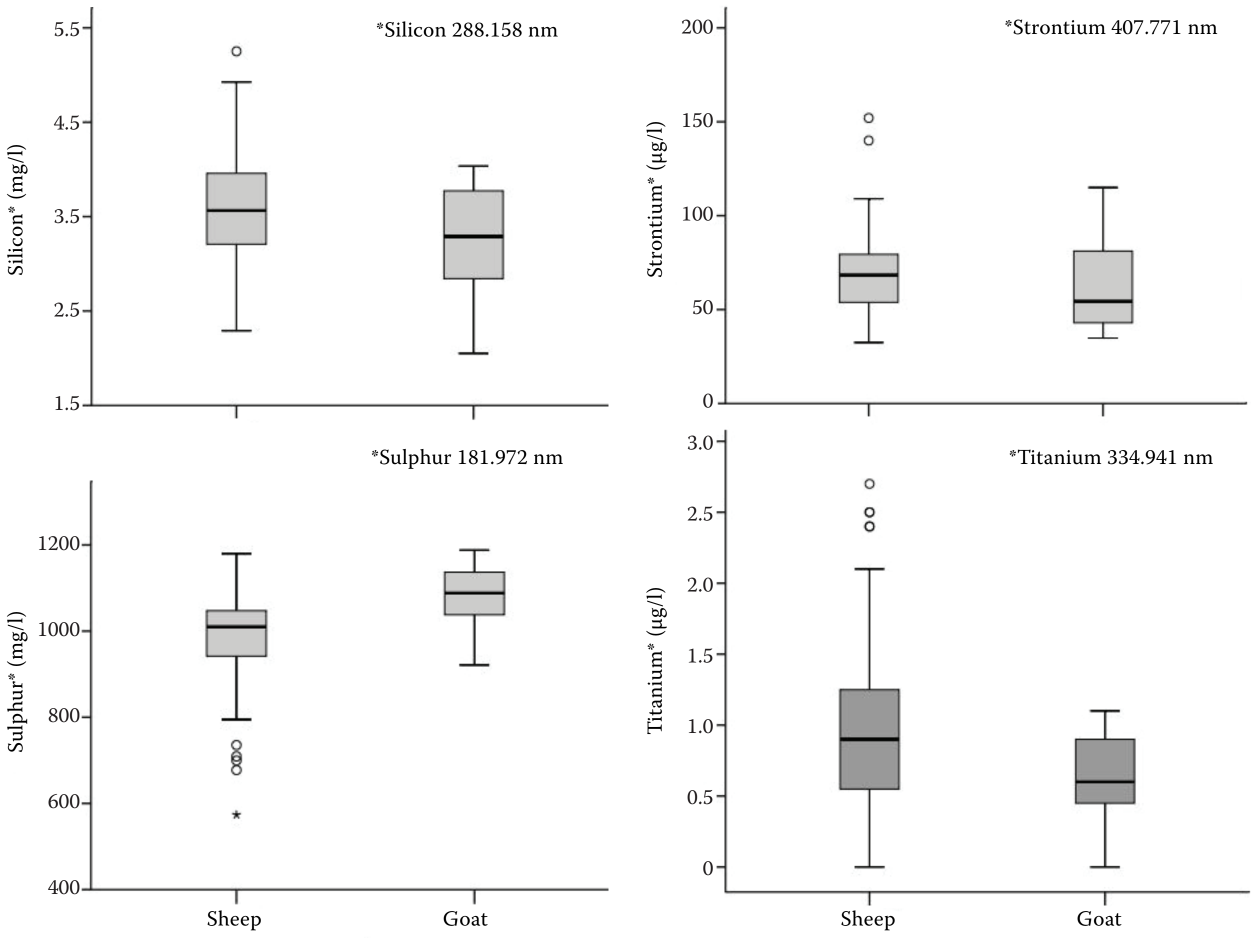

Figure 4. Boxplots of trace elements without reference values from selected literature and the laboratory (Idexx Vet Med Labor GmbH, Ludwigsburg, Germany) including results of serum blood samples of 75 sheep and 20 goats (except for Ti (n = 75 sheep, n = 19 goats)); black line in grey box = median, grey box = upper/lower quartile, whisker = minimum/maximum, ○/★ = outlier

ent values were provided, for As only one reference value was available from the laboratory.

Goats

Available reference values of macrominerals and trace elements for goats are summarised in Table 3, together with descriptive statistics of the test results of the study population. The classification of the supply with macrominerals and trace elements based on different reference values from the selected literature and the laboratory is provided in Table 4. For the elements Ca, Fe and Se there was a high agreement for the degree of supply between different reference values. In summary, 95% of the goats in the tested population were adequately supplied with Ca and, similarly, 85% had an adequate supply with Se. According to the reference values, 95% of goats had an oversupply of Fe. K and Mg were adequately supplied, but not according to all published reference values for these elements. Results from goats were compared with reference values for Cu, but there was no agreement between the references for supply with Cu.

DISCUSSION

Generally, the assessment of the macromineral and trace element status of farm animals is a valuable tool in the herd health management of flocks and individual animals. Below-par production and reproductive performance is one scenario in which assessment of mineral status in ruminants can be useful. More generally, it is important to avoid deficiencies, oversup-

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Table 1. Reference values for elements from selected literature, laboratory reference values and descriptive statistics (mean ($\bar{x}$) ± SD) of serum blood samples of tested sheep (n = 75)

Element	Author 1	Author 2	Author 3	Author 4	Laboratory	Study population		
						$\bar{x}$ ± SD	min.	max.
As (nmol/l)	na	na	na	na	<133.5[1,2]	9.8 ± 6.9	2.7	34.7
Ca (mmol/)	na	2.88–3.2[1]	2.2–3.0[1]	2.9–3.2[1]	2.25–3.0[1]	2.6 ± 0.3	2.0	3.1
Co (nmol/l)	3.0–34.0[1]	170.0–510.0[1]	na	na	na	8.7 ± 6.4	1.5	41.6
Cu (µmol/l)	11.8–26.8[1]	11.0–20.5[2]	10.0–12.0[2]	9.13–25.2[1]	9.0–12.0[1]	11.7 ± 2.8	3.3	19.2
Fe (µmol/l)	16.0–48.0[1]	30.0–40.0[1]	13.0–32.0[2]	29.7–39.7[1]	12.5–35.0[1]	32.1 ± 13.3	11.9	126.0
K (mmol/l)	na	3.9–5.4[1]	3.8–5.6[1]	3.9–5.4[1]	3.5–5.0[1]	6.5 ± 0.9	4.8	9.5
Mg (mmol/l)	na	0.9–1.26[1]	0.8–1.2[1]	0.9–1.2[1]	0.8–1.2[1]	1.1 ± 0.1	0.7	1.4
Mn (nmol/l)	18.2–109.2[1]	na	300.0–800.0[2]	na	na	37.7 ± 16.0	3.6	106.0
Mo (µmol/l)	0.01–0.52[1]	na	na	na	< 0.1[1]	1.8 ± 2.6	0.03	14.9
Se (µmol/l)	0.8–2.5[1]	1.0–6.3[1]	0.7–2.2[2]	1.4–2.0[1]	0.7–2.2[1]	1.0 ± 0.6	0.1	2.1
Zn (µmol/l)	8.4–18.4[1]	12.2–18.4[1]	na	12.2–30.6[1]	10.7–21.4[1]	10.2 ± 2.7	5.1	16.8
As (µg/l)	na	na	na	na	< 10[1,2]	0.7 ± 0.5	0.2	2.6
Ca (mg/l)	na	115–130[1]	88.2–120.2[1]	115–128[1]	90.2–120.2[1]	105.9 ± 10.1	78.0	124.0
Co (µg/l)	0.18–2[1]	10–30[1]	na	na	na	0.5 ± 0.4	0.1	2.5
Cu (mg/l)	0.75–1.7[1]	0.7–1.3[2]	0.64–0.76[2]	0.58–1.6[1]	0.57–0.76[1]	0.7 ± 0.2	0.2	1.2
Fe (µg/l)	900–2700[1]	1660–2220[1]	726–1787[2]	1620–2220[1]	700–1960[1]	1790.7 ± 743.8	662.0	7040.0
K (mg/l)	na	152–211[1]	149–219[1]	152–211[1]	136.8–195.5[1]	255.8 ± 36.2	187.0	371.0
Mg (mg/l)	na	22–28[1]	19.4–29.2[1]	22–28[1]	19.4–29.2[1]	26.5 ± 2.7	16.8	32.7
Mn (µg/l)	1–6[1]	na	16.5–44[2]	na	na	2.1 ± 0.9	0.2	5.8
Mo (µg/l)	1–50[1]	na	na	na	< 10[1]	174.3 ± 251.2	2.7	1430.0
Se (µg/l)	60–200[1]	80–500[1]	55.3–173.7[2]	110–160[1]	53–176[1]	76.1 ± 47.3	8.2	165.5
Zn (µg/l)	550–1200[1]	800–1200[1]	na	800–2000[1]	700–1400[1]	664.0 ± 173.2	330.0	1100.0

Author 1 = Herdt and Hoff (2011); Author 2 = Radostits et al. (2007); Author 3 = Baumgartner (2009); Author 4 = Pugh and Baird (2012); Laboratory = Idexx Vet Med Labor GmbH, Ludwigsburg, Germany; na = not available

[1]Reference values for serum

[2]Reference values for plasma

plies and nutritional maladies and to support animal welfare (Kincaid 2000; Humann-Ziehank et al. 2013).

Blood samples are usually used to determine the status of macrominerals and trace elements, because drawing blood is a minimally invasive procedure (Kincaid 2000; Herdt and Hoff 2011). According to Herdt and Hoff (2011) obtained results reflect the transport pool of an element. Kincaid (2000) reported that blood concentrations of trace minerals are lower in animals with signs of deficiency. Although blood samples are easy to collect, they do not allow sufficient sensitivity for the detection of the marginal status of trace elements (Kincaid 2000). Marginal deficiencies often remain undetected, because the transport pool and storage of trace elements in the body compensate until the deficiency is well advanced and clinical signs become visible (Kincaid 2000; Ramirez-Perez et al. 2000). Although marginal deficiencies are hard to detect, blood analyses in the context of herd monitoring can indicate feeding problems, particularly for the elements Mo, Se, I and Li (Haenlein and Anke 2011), before marginal status can be detected or before clinical signs become obvious. Alternatives to blood samples are specimens of liver, kidneys, muscle tissue, ribs, e.g. for Zn, Pb, and hair for Mo, Se, I and Li (Haenlein and Anke 2011; Pavlata et al. 2011).

The liver represents the best endogenous store of many trace elements and, thus, levels in this organ reflect overall organismal status (Kincaid 2000). Haenlein and Anke (2011) concluded that the liver is an indicator tissue for the detection of As, Cu, I, Mn, Mo and Pb deficiencies. In our study, all goats were deficiently supplied with the trace element Mn

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Table 2. Classification of the supply with macrominerals and trace elements of serum blood samples of tested sheep (n = 75) according to reference values from selected literature and the laboratory; values are given in percents

Element		Author 1	Author 2	Author 3	Author 4	Lab
	deficiency					0.0
As	adequate	na	na	na	na	100.0
	oversupply					0.0
	deficiency		77.3	2.7	77.3	2.7
Ca	adequate	na	22.7	88.0	22.7	88.0
	oversupply		0.0	9.3	0.0	9.3
	deficiency	5.3	100.0			
Co	adequate	93.3	0.0	na	na	na
	oversupply	1.3	0.0			
	deficiency	56.0	41.3	20.0	10.7	10.7
Cu	adequate	44.0	58.7	41.3	89.3	50.7
	oversupply	0.0	0.0	38.7	0.0	38.7
	deficiency	1.3	41.3	1.3	36.0	1.3
Fe	adequate	97.3	44.0	54.7	49.3	65.3
	oversupply	1.3	14.7	44.0	14.7	33.3
	deficiency		0.0	0.0	0.0	0.0
K	adequate	na	9.3	14.7	9.3	5.3
	oversupply		90.7	85.3	90.7	94.7
	deficiency		2.7	1.3	2.7	1.3
Mg	adequate	na	69.3	82.7	69.3	82.7
	oversupply		28.0	16.0	28.0	16.0
	deficiency	5.3		100.0		
Mn	adequate	94.7	na	0.0	na	na
	oversupply	0.0		0.0		
	deficiency	0.0				0.0
Mo	adequate	37.3	na	na	na	10.7
	oversupply	62.7				89.3
	deficiency	44.0	60.0	44.0	72.0	41.3
Se	adequate	56.0	40.0	56.0	22.7	58.7
	oversupply	0.0	0.0	0.0	5.3	0.0
	deficiency	29.3	81.3		81.3	60.0
Zn	adequate	70.7	18.7	na	18.7	40.0
	oversupply	0.0	0.0		0.0	0.0

Author 1 = Herdt and Hoff (2011); Author 2 = Radostits et al. (2007); Author 3 = Baumgartner (2009); Author 4 = Pugh and Baird (2012); Lab = Idexx Vet Med Labor GmbH, Ludwigsburg, Germany; na = not available

compared with the reference value from the laboratory and Haenlein and Anke (2011). This indicates that blood is not a good indicator tissue for evaluating the Mn status of animals. However, taking samples from the liver is an invasive and seldom practiced method. Trace element concentrations can also be measured in hair. Hair can be easily collected and stored for some time and, further, trace elements are accumulated in hair in higher concentrations than in blood (Combs et al. 1982), which is highly advantageous for the measurement of Mo and I (Haenlein and Anke 2011). Concentrations of trace minerals in hair, however, are biased by breed, age, colour of the hair and contamination. Additionally, urine, faeces, sweat and feed can affect results as secondary contamination (Combs et al. 1982). Furthermore, hair growth is not continuous and depends on, e.g. the season, body region and species (Combs 1987). For this reason, Kincaid (2000) concluded that hair responds too slowly to changes in the concentration of trace minerals.

In general, several factors other than inadequate amount or supply have an impact on the concentration of macrominerals and trace elements. For example, species, breed, sex, age, physiological status (e.g. stage of pregnancy and lactation), inflammation, antagonists, concentration in the forage (i.e. forage species, age of plant, soil type, climate, management practices), and season affect the bioavailability of minerals and can lead to a secondary deficiency (Ramirez-Perez et al. 2000; Khan et al. 2007; Russell and Roussel 2007; Khan et al. 2008; Herdt and Hoff 2011). For example, the efficiency of Cu absorption is greatly influenced by other interfering elements, especially Mo and S, which act as antagonists, but also Ca, Fe, Pb and Zn (Spears 2003; Radostits et al. 2007; Herdt and Hoff 2011). Furthermore, trace elements are able to work as antioxidants or affect the immunity of ruminants. Primarily Se, together with other elements such as Zn or Cu, is known for its activity against reactive oxygen forms in tissues. Additionally, Co, Cu, Fe, Se and Zn deficiencies reduce resistance to infection and increase susceptibility to infectious maladies (Spears 2000; Bhaskaram 2002; Misurova et al. 2009). Biological availability and absorption of minerals also differ between ruminants and non-ruminants (Spears 2003), and between ruminants, e.g. Mo levels are least tolerated by cattle, followed by sheep, but are tolerated by goats (Haenlein and Anke 2011). Concentrations of vitamin B12 rise in times of Co supply and fall during periods of dietary deficiency, because Co is needed for the production of vitamin B12 by ruminant bacteria (Smith and Marston 1970; Suttle 2005).

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Table 3. Reference values for elements from selected literature, laboratory reference values and descriptive statistics (mean ($\bar{x}$) ± SD) of serum blood samples of tested goats (n = 20)

Element	Author 1	Author 2	Author 3	Author 4	Laboratory	Study population		
						$\bar{x}$ ± SD	min.	max.
As (nmol/l)	na	na	na	na	< 133.5[1,2]	5.8 ± 11.7	4.0	8.0
Ca (mmol/)	na	na	2.2–2.8[1]	2.2–2.9[1]	2.2–3.0[1]	2.5 ± 0.2	2.1	2.7
Co (nmol/l)	na	na	na	na	na	10.5 ± 5.6	3.1	26.6
Cu (µmol/l)	na	na	13.0–17.0[2]	na	12.6–17.5[1]	13.0 ± 2.7	8.4	19.5
Fe (µmol/l)	na	na	23.0–25.0[2]	na	23.0–25.0[1]	33.8 ± 7.5	18.8	48.5
K (mmol/l)	na	na	4.9–6.1[1]	3.5–6.7[1]	3.5–6.7[1]	5.9 ± 0.7	4.8	7.0
Mg (mmol/l)	na	na	0.8–1.1[1]	1.2–1.5[1]	1.1–1.5[1]	1.2 ± 0.1	1.1	1.4
Mn (nmol/l)	na	na	100.0–200.0[2]	na	na	48.6 ± 13.9	27.3	91.0
Mo (µmol/l)	na	na	na	na	na	0.5 ± 0.6	0.02	2.3
Se (µmol/l)	na	na	0.8–2.0[2]	na	0.8–2.0[1]	1.0 ± 0.2	0.6	1.3
Zn (µmol/l)	na	na	na	na	10.0–19.0[1]	9.0 ± 3.6	3.6	16.7
As (µg/l)	na	na	na	na	< 10[1,2]	0.4 ± 0.1	0.3	0.6
Ca (mg/l)	na	na	88.2–112.2[1]	89–117[1]	88.2–120.2[1]	101.3 ± 6.3	83.6	110.0
Co (µg/l)	na	na	na	na	na	0.6 ± 0.3	0.2	1.6
Cu (mg/l)	na	na	0.83–1.08[2]	na	0.8–1.11[1]	0.8 ± 0.2	0.5	1.2
Fe (µg/l)	na	na	1284–1396[2]	na	1280–1410[1]	1886.5 ± 416.3	1050.0	2710.0
K (mg/l)	na	na	192–238[1]	137–262[1]	136.8–262[1]	232.1 ± 25.8	186.0	272.0
Mg (mg/l)	na	na	19.4–26.7[1]	28–36[1]	26.7–36.5[1]	39.3 ± 2.6	25.8	34.7
Mn (µg/l)	na	na	5.5–11[2]	na	na	2.7 ± 0.8	1.5	5.0
Mo (µg/l)	na	na	na	na	na	43.4 ± 58.3	2.2	219.0
Se (µg/l)	na	na	63.2–157.9[2]	na	62–158[1]	79.2 ± 13.4	50.9	99.7
Zn (µg/l)	na	na	na	na	654–1243[1]	587.4 ± 236.0	273.0	1090.0

Author 1 = Herdt and Hoff (2011); Author 2 = Radostits et al. (2007); Author 3 = Baumgartner (2009); Author 4 = Pugh and Baird (2012); Laboratory = Idexx Vet Med Labor GmbH, Ludwigsburg, Germany; na = not available

[1]Reference values for serum

[2]Reference values for plasma

Beside all these factors, which have an impact on the concentration macrominerals and trace elements in body tissues, the reliability of reference values is another variable which warrants discussion. Poppenga et al. (2012) claimed that reference values for feral animals have not been determined, although the development of specific reference values would be important for assessing population health. According to Russell and Roussel (2007), a reference value is based on samples obtained from healthy animals and represents 95% of the healthy population. However, the established reference values from the selected literature and the laboratory compared in this study were not identical and showed differences of varying magnitudes. For example, for the elements Co, Cu, Fe, Mn, Se and Zn measured in the serum of sheep and for Ca, Cu, Fe, Mg and Se measured in goats, the reference values from the selected literature differed. This makes it difficult to give definitive statements on the degree of supply in the study population. For Ca, K and Mg reference values for sheep are similar and a clear conclusion is possible. Haenlein and Anke (2011) also reported reference values for Cu and Zn in goats. Comparing our results with their reference value for Zn, the classification of the supply is similar to the classification from the laboratory (Table 4). Conversely, the reference value for Cu shows a wider range compared with the laboratory reference value and classification reveals that 25% of goats are oversupplied and 75% are adequately supplied with Cu (Table 4). Nonetheless, when the results of a given study are compared with reference values from different sources, the test meth-

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Table 4. Classification of the supply with macrominerals and trace elements of serum blood samples of tested goats (n = 20) according to reference values from selected literature and the laboratory; values are given in percents

Element		Author 1	Author 2	Author 3	Author 4	Lab
	deficiency					0.0
As	adequate	na	na	na	na	100.0
	oversupply					0.0
	deficiency			5.0	5.0	5.0
Ca	adequate	na	na	95.0	95.0	95.0
	oversupply			0.0	0.0	0.0
	deficiency			50.0		35.0
Cu	adequate	na	na	45.0	na	60.0
	oversupply			5.0		5.0
	deficiency			5.0		5.0
Fe	adequate	na	na	0.0	na	0.0
	oversupply			95.0		95.0
	deficiency			5.0	0.0	0.0
K	adequate	na	na	45.0	90.0	90.0
	oversupply			50.0	10.0	10.0
	deficiency			0.0	30.0	20.0
Mg	adequate	na	na	25.0	70.0	80.0
	oversupply			75.0	0.0	0.0
	deficiency			100.0		
Mn	adequate	na	na	0.0	na	na
	oversupply			0.0		
	deficiency			15.0		15.0
Se	adequate	na	na	85.0	na	85.0
	oversupply			0.0		0.0
	deficiency					75.0
Zn	adequate	na	na	na	na	25.0
	oversupply					0.0

Author 1 = Herdt and Hoff (2011); Author 2 = Radostits et al. (2007); Author 3 = Baumgartner (2009); Author 4 = Pugh and Baird (2012); Lab = Idexx Vet Med Labor GmbH, Ludwigsburg, Germany; na = not available

ods used have to be considered. Ideally, only results measured by a single laboratory using the same testing method should be compared and discussed with reference values from the laboratory. In our study, we had to compare our results with reference values from the literature, because the laboratory did not provide reference values for some elements. These textbook references were selected because they represent scientific literature specific for sheep and goats, which is freely available, also to practitioners in Austria.

Overall, the supply with minerals, especially of goats, was difficult to assess. The reasons for undersupplies, oversupplies and outliers are hard to determine. In our study we considered that all animals were clinically sound and none of the animals in the study showed obvious signs of toxicity or deficiency of any of the tested elements. Additionally, it has to be considered that the reliability of the detection of feeding deficiencies varies depending on the tissue that is tested. According to Haenlein and Anke (2011), blood tests have poor reliability for some trace elements, e.g. Zn and Cd. In our study, Cd was under the detection limit in all of the 95 blood serum samples. For Zn, the most suitable tissues are rib bones (Haenlein and Anke 2011). Nonetheless, blood serum samples are the most practical tool for evaluation of the Zn status in living animals. Although Zn deficiencies occur before a decline in blood concentration is detectable, low serum concentrations are an important symptom of undersupply with Zn (Herdt and Hoff 2011).

According to some authors, the concentration of elements in blood is also influenced by diet and highly affected by the nutritional content of feed (Ramirez-Perez et al. 2000, Yokus et al. 2004). Therefore, feed analyses can be informative for determining the causes of over- and undersupplies. Furthermore, a large number of tested animals and a homogenous group of study animals are required, and it is also necessary to consider physiological factors such as lactation or pregnancy. In the future, it will be important to conduct more studies with goats to generate new reference values particularly for trace minerals, as existing reference values sometimes show great differences.

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REFERENCES

Baumgartner W (ed.) (2009): Clinical Propaedeutics of House Animals and Pets (in German). 7th edn. Parey, Stuttgart. xiv+525 p.

doi: 10.17221/243/2015-VETMED

Bhaskaram P (2002): Micronutrient malnutrition, infection, and immunity: An overview. Nutrition Reviews 60, 40–45.

Combs DK (1987): Hair analysis as an indicator of mineral status of livestock. Journal of Animal Science 65, 1753–1758.

Combs DK, Goodrich RD, Meiske JC (1982): Mineral concentrations in hair as indicators of mineral status: a review. Journal of Animal Science 54, 391–398.

Fouda TA, Youssef MA, El-Deeb WM (2011): Correlation between zinc deficiency and immune status of sheep. Veterinary Research 4, 50–55.

Fraga CG (2005): Relevance, essentiality and toxicity of trace elements in human health. Molecular Aspects of Medicine 26, 235–244.

Haenlein GFW, Anke M (2011): Mineral and trace element research in goats: A review. Small Ruminant Research 95, 2–19.

Hefnawy AEG, Tortora-Perez JL (2010): The importance of selenium and the effects of its deficiency in animal health. Small Ruminant Research 89, 185–192.

Herdt TH, Hoff B (2011): The use of blood analysis to evaluate trace mineral status in ruminant livestock. The Veterinary Clinics of North America: Food Animal Practice 27, 255–283.

Hostetler CE, Kincaid RL, Mirando MA (2003): The role of essential trace elements in embryonic and fetal development in livestock. Veterinary Journal 166, 125–139.

Humann-Ziehank E, Ganter M, Hennig-Pauka I, Binder A (2008): Trace mineral status and liver and blood parameters in sheep without mineral supply compared to local roe deer (Capreolus capreolus) populations. Small Ruminant Research 75, 185–191.

Humann-Ziehank E, Tegtmeyer PC, Seelig B, Roehrig P, Ganter M (2013): Variation of serum selenium concentrations in German sheep flocks and implications for herd health management consultancy. Acta Veterinaria Scandinavica 55, 82.

Khan ZI, Hussain A, Ashraf M, Ashraf MY, McDowell LR (2007): Macromineral status of grazing sheep in a semi-arid region of Pakistan. Small Ruminant Research 68, 279–284.

Kincaid RL (2000): Assessment of trace mineral status of ruminants: a review. Journal of Animal Science 77, 1–10.

McDowell LR (ed.) (2003): Minerals in Animal and Human Nutrition. 2nd edn. Elsevier Science, Amsterdam. xvi + 644 pp.

Misurova L, Pavlata L, Pechova A, Dvorak R (2009): Selenium metabolism in goats – Maternal transfer of selenium to newborn kids. Veterinarni Medicina 54, 125–130.

Moritz A (ed.) (2014): Clinical Laboratory Diagnostics in Veterinary Medicine (in German). 7th edn. Schattauer, Stuttgart. xxii + 934 pp.

Or ME, Kayar A, Kiziler AR, Parkan C, Gonul R, Bora Barutcu B, Dodurka HAT (2005): Determination of levels of some essential (iron, copper, zinc) and toxic (lead, cadmium) metals in the blood of sheep and in samples of water, plants and soil in Northwest Turkey. Veterinarski Arhiv 75, 359–368.

Pavlata L, Chomat M, Pechova A, Misurova L, Dvorak R (2011): Impact of long-term supplementation of zinc and selenium on their content in blood and hair in goats. Veterinarni Medicina 56, 63–74.

Poppenga RH, Ramsey J, Gonzales BJ, Johnson CK (2012): Reference intervals for mineral concentrations in whole blood and serum of bighorn sheep (Ovis canadensis) in California. Journal of Veterinary Diagnostic Investigation 24, 531–538.

Pugh DG, Baird AN (eds) (2012): Sheep and Goat Medicine. 2nd edn. Saunders Elsevier, Maryland Heights. xiv+621 pp.

Radostits OM, Gay CC, Hinchcliff KW, Constable PD (eds) (2007): Veterinary Medicine: a Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats. 10th edn. Saunders Elsevier, Edinburgh. xxii + 2156 p.

Ramirez-Perez AH, Buntinx SE, Rosiles R (2000): Effect of breed and age on the voluntary intake and the micromineral status of non-pregnant sheep. II. Micromineral status. Small Ruminant Research 37, 231–242.

Rankins Jr DL, Pugh DG (2012): Feeding and nutrition. In: Pugh DG, Baird AN (eds): Sheep and Goat Medicine. 2nd edn. Saunders Elsevier, Maryland Heights. 18–49.

Russell KE, Roussel AJ (2007): Evaluation of the Ruminant Serum Chemistry Profile. Veterinary clinics of North America: Food Animal Practice 23, 403–426.

Smith RM, Marston HR (1970): Production, absorption, distribution and excretion of vitamin B 12 in sheep. British Journal of Nutrition 24, 857–877.

Spears JW (2000): Micronutrients and immune function in cattle. The Proceedings of the Nutrition Society 59, 587–594.

Spears JW (2003): Trace mineral bioavailability in ruminants. The Journal of Nutrition 133, 1506–1509.

Suttle N (2005): Assessing the needs of sheep for trace elements. In Practice 27, 474–483.

Sykes AR (2007): 53. Deficiency of mineral macro-elements. In: Aitken ID (ed.): Diseases of Sheep. 4th edn. Blackwell Publishing, Oxford. 363–377.

Yokus B, Cakir DU, Kurt D (2004): Effects of seasonal and physiological variations on the serum major and trace element levels in sheep. Biological Trace Element Research 101, 241–255.

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