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## **Genetic and environmental factors of macroelements concentration in blood serum and osseous tissue of lambs in north-west Poland**

### Summary

The aim of the study was to determine the concentration of Na, K, Ca, P<sub>ig</sub> and Mg in blood serum and osseous tissue of the lambs of various breeds at age of 14 days. The studies were carried out on 221 lambs in various sheepfolds in Northwest Poland. The material for the studies comprised the blood serum and osseous tissue ash of the 4th and 5th caudal vertebrae. The values of macroelement concentrations in osseous tissue were expressed in mmol/l in 1g of dry matter. The highest ( $P \leq 0.01$  and  $P \leq 0.05$ ) Na concentration was found in the serum of Ile de France lambs ( $152.31 \text{ mmol/l} \pm 7.50$ ), of K and P<sub>ig</sub> in the Kent lambs ( $5.52 \text{ mmol/l} \pm 0.48$ ;  $3.37 \text{ mmol/l} \pm 0.51$ ), and Ca and Mg — in the Polish Merino lambs ( $2.42 \text{ mmol/l} \pm 0.27$ ;  $1.25 \text{ mmol/l} \pm 0.09$ ). The Ca/P ratio was similar ( $0.70 \pm 0.09$ ), whereas the Ca/Mg ratio was the highest for the Polish Merino lambs ( $2.35 \pm 0.27$ ). Osseous tissue Na concentration was similar ( $2.25 \text{ mmol/l} \pm 0.43$ ). The highest osseous tissue concentration of K was found in the lambs: Kent and Polish Merino ( $1.29 \text{ mmol/l} \pm 0.23$ ) and Ile de France ( $1.36 \text{ mmol/l} \pm 0.27$ ). The lowest Ca was recorded in the lambs Kent and Merino ( $9.50 \text{ mmol/l} \pm 1.72$ ), the highest — in Blackheaded ( $19.74 \text{ mmol/l} \pm 2.02$ ). The lowest P<sub>ig</sub> concentration was found in the osseous tissue of the Polish Merino ( $9.70 \text{ mmol/l} \pm 1.76$ ). The concentration of magnesium ranged between  $0.83 \text{ mmol/l} \pm 0.11$  in the serum of Kent lambs and  $1.02 \text{ mmol/l} \pm 0.15$  in the serum of the Leine lambs. The lowest Ca/P ratio was observed in the osseous tissue of the Kent lambs ( $0.50 \pm 0.10$ ), whereas the highest was found in the Ile de France lambs ( $1.17 \pm 0.17$ ). A wide range ( $P \leq 0.01$ ) of the Ca/Mg ratio were recorded, from  $11.40 \pm 1.72$  in Kent to  $20.37 \pm 2.09$  in Blackheaded. Basing on the concentrations of Na, K, Ca, P<sub>ig</sub> and Mg in the blood serum of 14-day-old lambs, no conclusions should be drawn as to the content of the elements in the osseous tissue.

Key Words: lambs, macroelements, serum, bone tissue

### Zusammenfassung

Titel der Arbeit: **Genetischer und umweltbedingter Einfluss auf den Makroelementgehalt im Blutserum und Knochengewebe von Lämmern im nordwestlichen Polen**

Das Ziel der Untersuchungen war die Bestimmung des Gehaltes an Na, K, Ca, P<sub>ig</sub> i Mg im Blutserum und Knochengewebe der Lämmern verschiedener Rassen im Alter von 14 Tagen. Für die Untersuchungen stand eine Gruppe von 221 Lämmern aus den Herden im nordwestlichen Polen zur Verfügung. Untersucht wurde Blutserum und die Asche des Knochengewebes des 4. und 5. Schwanzwirbels. Die ermittelten Ergebnisse im Knochengewebe wurden in mmol/l je 1 g TM ausgedrückt. Im Blutserum wurde die größte Na-Konzentration ( $p \leq 0,01$  und  $p \leq 0,05$ ) bei den Lämmern der Rasse Ile de France ( $152,31 \text{ mmol/l} \pm 7,50$ ) festgestellt, die K- i P<sub>ig</sub> -Konzentration war bei der Rasse Kent ( $5,52 \text{ mmol/l} \pm 0,48$ ;  $3,37 \text{ mmol/l} \pm 0,51$ ), und Ca- i Mg-Gehalt beim Polnischen Merinoschaf ( $2,42 \text{ mmol/l} \pm 0,27$ ;  $1,25 \text{ mmol/l} \pm 0,09$ ) am größten. Das Ca-P-Verhältnis wies bei allen Rassen etwa gleiche Werte ( $0,70 \pm 0,09$ ) auf und die größten Werte des Ca-Mg-Verhältnisses wurden bei den Lämmern der Rasse Polnisches Merinoschaf ( $2,35 \pm 0,27$ ) festgestellt. Der Na-Gehalt im Knochengewebe lag bei allen Rassen auf annähernd gleichem Niveau ( $2,25 \text{ mmol/l} \pm 0,43$ ). Der höchste K-Gehalt im Knochengewebe wurde bei den Lämmern der Rassen Kent und Merino ( $1,29 \text{ mmol/l} \pm 0,23$ ) sowie Ile de France ( $1,36 \text{ mmol/l} \pm 0,27$ ) festgestellt. Der niedrigste Ca-Gehalt wurde bei den Lämmern der Rassen Kent und Merino ( $9,50 \text{ mmol/l} \pm 1,72$ ) und der höchste bei Schwarzköpfigen Fleischschafen ( $19,74 \text{ mmol/l} \pm 2,02$ ) beobachtet. Der P<sub>ig</sub> -Gehalt war im Knochengewebe von Merinoschafen ( $9,70 \text{ mmol/l} \pm 1,76$ ) am niedrigsten. Der Mg-Gehalt im Blutserum lag im Bereich von  $0,83 \text{ mmol/l} \pm 0,11$  bei den Lämmern der Rasse Kent bis  $1,02 \text{ mmol/l} \pm 0,15$  bei der Rasse Leine. Im Knochengewebe der Kent-Lämmern wurde das niedrigste ( $0,50 \pm 0,10$ ) und bei der Rasse Ile de France das

höchste ( $1,17 \pm 0,17$ ) Ca-P-Verhältnis festgestellt. Das Ca-Mg-Verhältnis im Knochengewebe schwankte in einem breiten Bereich ( $p \leq 0,01$ ): von  $11,40 \pm 1,72$  bei der Rasse Kent bis  $20,37 \pm 2,09$  beim Schwarzköpfigen Fleischschaf. Aufgrund der Na-, K-, Ca-,  $P_{ig}$ - und Mg-Konzentration im Blutserum der Lämmer im Alter von 14 Tagen kann nicht über den Gehalt an diesen Makroelementen im Knochengewebe geschlussfolgert werden.

Schlüsselwörter: Lämmer, Makroelemente, Blutserum, Knochengewebe

### Introduction

The monitoring against mineral deficits during both pre-parturient and post-parturient periods has been carried on in Poland as standard examinations based on blood analyses and/or clinical syndrome recognition (BARANOWSKI et al., 2000a; BRZOSTOWSKI et al., 1995; WOLAŃCZYK-RUTKOWIAK, 1986). However, the analyses of food and the concentration of elements in the serum or plasma of adult animals (KORMAN, 1999) give insufficient results for a comprehensive evaluation of livestock animals as far as mineral components are concerned. This additionally requires evaluation of young animals in particular stages of growth and development (SABA and BIAŁKOWSKI, 1988). For this purpose, the element accumulation is determined in the following materials: colostrum or milk (JELINEK et al., 1996; MIGDAŁ et al., 1990), hair (KOŚLA et al., 1989) and wool (SZTYCH and SOROCZYŃSKA, 1994), or by means of tissue- or organ-specific analyses (FALANDYSZ and LORENC-BIAŁA, 1988; NOWAKOWSKI, 1989). Live examinations of muscles or liver require tissue biopsies, whereas the composition of brain can only be evaluated *post mortem*. Those organs are most frequently examined for the concentration of trace elements in the studies on the impact of environment pollutants (ENNA et al., 1989) or their interactions that affect animals (PURDEY, 2000).

Bone tissue of lamb tails is a good object for live estimation of mineralisation degree of osseous system, as the tissue retains mineral elements accumulated during prenatal period. During neonatal development, tail osseous tissue serves as a wealthy store of minerals (BARANOWSKI et al. 1999; BARANOWSKI et al., 2000b; KLATA et al., 2000). Owing to the assessment of osseous tissue mineral composition during the initial stages of life, it is easier to decide whether the food of the growing lambs should be supplemented with minerals or, on the contrary, mineral supplementation should be restricted. The aim of this study was to measure the concentration of Na, K, Ca,  $P_{ig}$ , and Mg in the blood serum and osseous tissue of lambs at 14 days of age.

### Material and methods

The analyses covered 221 lambs of 5 breeds (Kent,  $n = 51$ ; Leine,  $n = 54$ ; Polish Merino,  $n = 50$ ; Blackheaded  $n = 27$ ; Ile de France,  $n = 39$ ). The Leine sheep were kept in buildings with limited access to daylight. The sheep of the remaining breeds had unrestricted access to ambient light all year round. The feeding of the ewes in all the herds was similar and was based on farm feeds. The daily ration included green forage, meadow hay, semi-sugar beet, carrot, maize and sugar beet leave silage, summer and winter corn straw, oats and lupine grain in 0.20-0.25 kg per head daily. The feeding systems in all the herds were similar, based on Livestock Animal Feeding Standards by the Polish Institute of Animal Husbandry. The quantity of dry matter, oat equivalents and total digestible protein in the ration was modified only by reason of

the body weight of the ewe before tapping season, average herd reproductive performance in the previous reproductive season and during the period of foetal growth and development. During the period preceding the tapping season as well as in pregnancy, the ewes had unrestricted access to water and salt licks.

The sheep of the Leine (Cerkwica – 54°00'N; 15°05'E), Kent, Blackheaded, and Polish Merino (Nowielice – 53°59'N; 15°18'E) were mated from 21 September (92 days after summer solstice). The service of the Ile de France females (Kosin – 53°07'N; 15°03'E) was begun from 20 August, when the first symptoms of heat were observed at more than 50% of the ewes. The mean temperature in March, in the rooms where the lambs were born, remained between 11°C and 14°C. The lambs of Ile de France were born end of January and beginning of February, and the interior temperature ranged between 2°C and 5°C.

Blood and bone tissue of tail vertebrae were collected from 14-day-old ( $\pm 2$  days) lambs, which had been born in the peak lambing season. The erythrocytic concentration of potassium in the lambs that were selected for the studies was lower than 27 mmol/l (BARANOWSKI, 1998). Until the sampling (collection of blood and caudal vertebrae), the lambs were fed only on the mothers' milk. No material was collected from the lambs that received additional feed or those with diarrhoea. On the day preceding blood and bone tissue collection, the lambs were weighed to 0.1 kg. In the laboratory, the blood was centrifuged at 3500 rpm for 20 min, and the 4th and 5th vertebrae were prepared for analyses. The isolated vertebrae were thoroughly cleaned of soft tissues, weighed, and stored at 25°C in exicator until the analyses were done. In the blood serum, the concentration of the following macroelements was determined: sodium, potassium, and calcium (flame photometry), inorganic phosphorus (Fiske-Subbarow method), and magnesium (atomic absorption spectrophotometer). Before the same minerals were assayed in the bone tissue, the vertebrae had been dried to constant mass in porcelain casserole dishes at 105°C. After the drying, the vertebrae were combusted at 600°C, and then the following procedure was applied: (1) 10 ml of distilled water + 1 ml of concentrated HCl was added to the casserole dish, which was next placed in sand bath at 600°C and boiled until the ash was entirely diluted; (2) after cooling, the dish content was percolated into a 100-ml volumetric flask, the dish and the filter being washed several times with distilled water. Next, the filter was placed in the casserole dish and burnt in the sand bath, being subsequently combusted in a furnace at 600°C. The ash was then diluted (5 ml H<sub>2</sub>O and 3 drops of concentrated HCl); next the solution was boiled and, after cooling, poured into a 100-ml volumetric flask. The flask was filled up with distilled water to 100 ml. The solution prepared this way was ready for further analyses. The results of this study are presented in SI units.

Because the age of the parents, the ewes' body weight before the tugging season, the sex of the lambs, and the size of the litter, in which they were born, did not represent a source of variability, the differences between the groups of lamb breeds were tested with one-way ANOVA using Scheffe's test. The Pearson's coefficients of correlation were calculated using Statistica 5.0 software package.

## Results

As a result of the studies, differences were observed among the two-week-old lambs of five breeds in the concentration of macroelements in blood serum (Table 1) and in tail vertebrae bone tissue (Table 2).

**Blood serum.** The highest concentration of sodium (152.31 mmol/l/  $\pm$  7.50) was recorded in the serum of Ile de France lambs (Table 1). The level of this concentration was higher ( $P \leq 0.01$ ) by 17.55 mmol/l compared to the level observed in the Leine lambs (134.76 mmol/l). The mean blood serum concentrations of sodium in the Kent, Polish Merino and Blackheaded lambs were similar.

Table 1

The content of macroelements (mmol/l) in blood serum of five breed lambs (Gehalt an Makroelementen im Blutserum bei Lämmern von fünf Schafrassen)

Macro-element	BREED of SHEEP									
	Romney Marsh* n=51		Leine n=54		Polish Merino* n=50		Blackheaded* n=27		Ile de France n=39	
	x	sd	x	sd	x	sd	x	sd	x	sd
Na	143.12 <sup>AB</sup>	7.99	134.76 <sup>ACDE</sup>	4.18	142.08 <sup>CF</sup>	10.11	143.18 <sup>DG</sup>	9.10	152.31 <sup>BEFG</sup>	7.50
K	5.52 <sup>ABC</sup>	0.48	5.01 <sup>ADE</sup>	0.41	4.96 <sup>BFG</sup>	0.47	5.48 <sup>DFH</sup>	0.41	4.58 <sup>CEGH</sup>	0.29
Ca	2.32 <sup>A</sup>	0.19	2.05 <sup>ABCD</sup>	0.14	2.42 <sup>Ba</sup>	0.27	2.27 <sup>Ca</sup>	0.11	2.31 <sup>D</sup>	0.15
P <sub>ig.</sub>	3.37 <sup>A</sup>	0.51	2.97 <sup>ABaC</sup>	0.34	3.36 <sup>a</sup>	0.25	3.27 <sup>a</sup>	0.34	3.32 <sup>C</sup>	0.32
Mg	1.03 <sup>Aa</sup>	0.12	1.00 <sup>BC</sup>	0.10	1.25 <sup>ABDE</sup>	0.09	0.98 <sup>DF</sup>	0.13	1.10 <sup>aCEF</sup>	0.11
Ca/P	0.70	0.11	0.70	0.10	0.72	0.08	0.70	0.07	0.70	0.07
Ca/Mg	2.28 <sup>AB</sup>	0.31	2.06 <sup>AC</sup>	0.21	1.94 <sup>BCDa</sup>	0.23	2.35 <sup>Db</sup>	0.27	2.12 <sup>ab</sup>	0.26

Explanation: means bearing the same superscripts are significantly different at – capital letters  $P \leq 0.01$ ; small letters  $P \leq 0.05$ .

\*The sheep lived in the same flock.

The highest blood serum concentration of potassium was found in the Kent lambs (5.52 mmol/l  $\pm$  0.48), whereas the lowest level was observed for the Ile de France lambs (4.58 mmol/l  $\pm$  0.29). In the scope of this trait, the Kent lambs differed significantly ( $P \leq 0.01$ ) from the Polish Merino and Ile de France lambs. It was observed that the Kent and Blackheaded lambs, whose mothers remained in the same environmental conditions, had a higher ( $P \leq 0.01$ ) blood serum potassium concentration compared to the mean blood serum level of this element of the Polish Merino lambs in the same sheepfold.

Contrary to blood serum concentration of potassium, the highest calcium concentration (2.42 mmol/l  $\pm$  0.27) appeared in the Polish Merino lambs, whereas the lowest (2.05 mmol/l  $\pm$  0.14) was observed in the serum of the Leine lambs. In the serum of the latter group, also the lowest concentration of inorganic phosphorus was recorded (2.97 mmol/l  $\pm$  0.34). Its value differed ( $P \leq 0.01$  and  $P \leq 0.05$ ) from those observed for the lambs of the remaining breeds.

The mean magnesium concentration in blood serum of the studied lambs ranged between 0.98 mmol/l  $\pm$  0.13 (Blackheaded) and 1.25 mmol/l  $\pm$  0.09 (Polish Merino).

The mean blood serum calcium/phosphorus ratio remained in the range 0.70–0.72, whereas the mean calcium/magnesium ratio ranged between 1.94  $\pm$  0.23 (Polish Merino) and 2.35  $\pm$  0.27 (Blackheaded).

**Bone tissue.** The mean bone tissue sodium concentration was nearly the same for all the lambs, ranging between 2.74 mmol/l  $\pm$  0.37 to 2.77 mmol/l  $\pm$  0.28.

The highest bone tissue concentration of potassium was observed for the Ile de France lambs (1.36 mmol/l in 1 g of bone tissue dry matter  $\pm$  0.27). The value of the concentration was higher ( $P \leq 0.01$ ) by 0.57 mmol/l compared to that of the Leine

lambs and by 0.59 mmol/l ( $P \leq 0.01$ ) compared to that of the Blackheaded lambs (Table 2).

Table 2

The content of mineral elements (mmol/l in 1 g dry weight) in bone tissue of caudal vertebra of five breed lambs (Gehalt an Mineralstoffen im Knochengewebe der Schwanzwirbel bei Lämmer von fünf Schafassen)

Macro-element	BREED of SHEEP									
	Romney Marsh*		Leine		Polish Merino*		Blackheaded*		Ile de France	
	n=51		n=54		n=50		n=27		n=39	
	x	sd	x	sd	x	sd	x	sd	x	sd
Na	2.74	0.37	2.77	0.61	2.74	0.37	2.76	0,23	2.77	0.28
K	1.29 <sup>AB</sup>	0.23	0.79 <sup>ACD</sup>	0.22	1.29 <sup>CE</sup>	0.23	0.77 <sup>BEF</sup>	0,11	1.36 <sup>DF</sup>	0.27
Ca	9.53 <sup>ABC</sup>	1.80	15.94 <sup>ADE</sup>	3.16	9.51 <sup>DFG</sup>	1.78	19.74 <sup>BEFH</sup>	2,02	14.62 <sup>CGH</sup>	3.12
P <sub>ig</sub>	19.16 <sup>ABC</sup>	1.78	16.47 <sup>AD</sup>	2.75	9.70 <sup>BDEF</sup>	1.76	17.42 <sup>Ea</sup>	4,84	15.21 <sup>Cfa</sup>	2.18
Mg	0.83 <sup>AB</sup>	0.11	1.02 <sup>ACD</sup>	0.15	0.84 <sup>CE</sup>	0.11	0.97 <sup>BE</sup>	0,01	0.89 <sup>D</sup>	0.10
Ca/P	0.50 <sup>ABCD</sup>	0.10	0.97 <sup>AE</sup>	0.15	1.06 <sup>BF</sup>	0.17	1.17 <sup>CEFG</sup>	0,17	0.96 <sup>DG</sup>	0.17
Ca/Mg	11.40 <sup>ABC</sup>	1.72	15.86 <sup>ADE</sup>	3.45	11.43 <sup>DFG</sup>	1.75	20.37 <sup>BEFH</sup>	2,09	16.39 <sup>CGH</sup>	3.02
B. w.(kg)	7.45	1.75	7.00	1.78	7.26	1.49	7.52	1,86	7.75	1.64
Ash (in g)	0.298 <sup>AaBC</sup>	0.02	0.268 <sup>AD</sup>	0.06	0.263 <sup>B</sup>	0.03	0.279 <sup>a</sup>	0,02	0.247 <sup>CD</sup>	0.04

Explanation: means bearing the same superscripts are significantly different at – capital letters  $P \leq 0.01$ ; small letters  $P \leq 0.05$ .

\* The sheep lived in the same flock

Contrary to bone tissue concentration of potassium, the highest concentration of calcium was found in the tissue of the Blackheaded lambs. Its mean value was higher ( $P \leq 0.01$ ) by 3.80 mmol/l compared to that of the Leine lambs (15.94 mmol/l  $\pm$  3.16). No differences in bone tissue concentration of calcium were found between the Leine and Ile de France lambs. On the other hand, calcium level in the Kent and Merino lambs was lower ( $P \leq 0.01$ ) by 10.22 mmol/l compared to the Blackheaded lambs, whose mothers were kept with the mothers of both former breeds.

The lowest inorganic phosphorus concentration (9.59 mmol/l  $\pm$  1.83) was found in the bone tissue of the Polish Merino lambs. This value differed significantly ( $P \leq 0.01$ ) from those of the remaining breeds. The highest level of the element was recorded in the Kent lambs, followed by the Blackheaded, Leine, and Ile de France.

The bone tissue of the Leine lambs had the highest magnesium concentration (1.02 mmol/l  $\pm$  0.15), which was higher ( $P \leq 0.01$ ) compared to those of the Merino, Leine, and Ile de France.

The Kent lambs had the lowest calcium to phosphorus ratio (0.50  $\pm$  0.10). The highest value of this index was found in the bone tissue of the Blackheaded lambs (1.17  $\pm$  0.17). The calcium/magnesium ratio of the latter breed was also the highest (20.37  $\pm$  2.09), and the value differed ( $P \leq 0.01$ ) from those of the other breeds.

Ash content is an important index of bone tissue mass of the lambs. The highest ash content (0.298 g  $\pm$  0.02) was found in the bone tissue of the Kent lambs, which differed ( $P \leq 0.05$  and  $P \leq 0.01$ ) from the remaining breeds. The lowest ash content in bone tissue was found in the Ile de France lambs (0.247 g  $\pm$  0.04), however their body weight at 14 days of age was the highest (7.75 kg  $\pm$  1.64).

## Discussion

The results of this study indicate that the breed of the lambs is a factor that forms the level of some macroelements in the blood serum and bone tissue during the neonatal period. What should be stressed, the highest sodium concentration in blood serum was found in the Ile de France lambs ( $152.31 \text{ mmol/l} \pm 7.50$ ), which differed from the Blackheaded ( $143 \text{ mmol/l} \pm 9.10$ ), and the Leine ( $134.76 \text{ mmol/l} \pm 4.18$ ) lambs. The lambs of Kent, Polish Merino, and Blackheaded were born in the same period and sheepfold, and the soils of the coastal region, where the sheepfolds Cerkwica and Nowielice are located, are the same. A probable, however not the only reason of the existing differences in blood serum sodium concentration of the lambs is the multi-generational adaptation of the animals to the high sodium concentration in the coastal environment. The forage in such regions contains much sodium, which comes from seawater aerosols (PASTRANA et al., 1991). The precipitating water increases sodium content in both soil and plants. Because the environment is rich in sodium, the sheep organism is not forced to economical management of the element, unlike the sheep that live in the areas far away from the seacoast. The sheepfold of the Ile de France lambs is situated about 200 km away from the Baltic coast. However, such explanation is not entirely consistent, as the bone tissue sodium concentration should in this case be probably also higher. It seems that also another interpretation should be taken into account, which cannot be ignored in the light of many reports.

In the herd of the Ile de France sheep, the lambs were born at the end of January, when the internal and ambient temperatures were low, much below the zone of thermoneutrality, which for the neonates of this species is  $28^{\circ}\text{C}$  (MOUNT, 1979). Therefore, the reasons for the high concentration of  $\text{Na}^+$  in blood serum of this group compared to the remaining groups may presumably be found in the effect of low temperature on the their organism and the adaptation to chill. The sheep neonates belong to livestock animals of the intermediate noradrenaline-tyroxine type of thermal stability, which is characterized by full activity of thyroïdal system at birth and which has the component necessary for both types of thermogenesis: trepidatory and non-trepidatory (ALEXANDER and WILLIAMS, 1968).

A two-week old lamb is characterised by a relatively high somatic maturity and extensive muscle activity. When the Ile de France lambs were born in the conditions much below the thermal comfort, biochemical reactions took place, controlled by the thyroïdal hormones. These reactions cover, among others, the metabolic paths related to oxidative phosphorylation, where  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  is induced, and  $\text{Na}^+$  transport energy is released, besides other undergoing processes. As a result, large quantities of heat are released. The energy is also released if the sodium pump is activated or if the cell-membrane permeability is increased for sodium and potassium along with synthesis and hydrolysis of ATP in a number of tissues and organs, i.e. liver, renal cortex, skeletal muscles, and heart (ASANO et al., 1976; ISMAIL-BEIGI and EDELMAN, 1970). As a result of low temperature, increased oxygen consumption occurs in brown adipose tissue, liver, kidneys, and muscles, which is accompanied by enhanced  $\text{Na}^+$  transport. Noradrenaline is the factor that activates the sodium pump of plasmatic membranes and that enhances the activity of  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  not only in brown adipose tissue, but in skeletal muscles as well (HOROWITZ, 1974). Although no differences were found in the concentration of sodium, the osseous tissue potassium

concentration of the Kent, Polish Merino, and Ile de France lambs was the highest (Table 1).

Among the studied elements, particular attention should be drawn to the concentration of calcium, inorganic phosphorus, and magnesium in the osseous tissue of the lambs. In the samples collected from the Kent and Polish Merino, calcium concentration was the lowest, whereas the level of inorganic phosphorus was the highest in the Kent, and the lowest in the Polish Merino lambs. The high osseous tissue concentration of inorganic phosphorus in the Kent lambs resulted in a low ratio of both elements, which from the point of view of mineralisation processes and subsequent normal mineral metabolism in the organisms of the lambs may lead to malformations of the limbs (ROGOWSKA and KOZŁOWSKA, 1986). Despite the low level of calcium and inorganic phosphorus in the osseous tissue of the Polish Merino lambs, the mean ratio of these elements belonged to the highest ones in this study, though non-optimal. It has been established that the Ca/P ratio in osseous tissue should be around like 2:1, however it is sometimes higher or lower. Due to the fact that the dams of both breeds remained throughout pregnancy at the same maintenance and feeding conditions with unrestricted access to the outside run, it can be assumed that such concentrations resulted from the genotypic differences between the lambs. Also, an interesting fact is that calcium and phosphorus concentrations were higher ( $P \leq 0.01$ ), and potassium concentration was lower ( $P \leq 0.01$ ) in the osseous tissue of the Leine lambs, compared to the values observed in both previously mentioned breeds. The herd of the Leine sheep remained in the coastal zone of the West Pomerania region, on sandy soils, about 20 km away from the location where the Kent and Polish Merino sheep were kept. The value of calcium concentration in the tissues is influenced by its content in the feed, but also by the element of movement and physical effort (OLIVERI et al. 1999), which was unavailable to the pregnant Leine ewes due to the management system.

The key role in the biological osseous tissue mineralisation belongs to specialised cells, which initiate and control the process. These cells are response to the activity of hormones and other mediators, as well as to changing environmental conditions, synthesise and secrete a range of substances controlling the course of the process and its result. Endoplasmatic reticule and mitochondria are responsible for the accumulation of calcium and phosphorus ions, and for their subsequent release to the cytoplasm (OSTROWSKI, 1995). During the embryonic development, the cell division is accompanied by the growth, morphogenesis, and differentiation of the embryo cells. As a result of mitotic divisions, the number of cells, cell nuclei, and mitochondria increases. For the mutton breeds of sheep, such as Blackheaded or Ile de France, the processes of growth and development in the prenatal period probably result in a more extensive growth of muscle tissue than osseous tissue, which is manifested by the mean ash mass and the mean body weight along with a higher proportion of calcium in the examined tissue. The lambs of the mutton Blackheaded breed had by 5.12 mmol/l higher ( $P \leq 0.01$ ) proportion of calcium in osseous tissue compared to the Ile de France lambs, whose body weight was the highest among the studied lambs. The coefficient of linear correlation between the body weight of the Blackheaded lambs and their ash content of osseous tissue ( $r = 0.410$ ) was statistically significant ( $P \leq 0.05$ ), whereas that for Ile de France was low and negative ( $r = -0.015$ ). For the remaining groups, these values were lower than  $r = 0.1$ .

In the studies on effect of calcium and sodium excessive content in diets (ROGOWSKA and KOZŁOWSKA, 1986), it was observed – basing on the mineral components balance for growing sheep – that an excess of Na improved the digestibility and retention of Mg, and a high concentration of Ca reduced the retention of P. This was considered as an explanation for the very good assimilability of magnesium and its high level in the blood serum of the Polish Merino lambs kept in the Baltic coastal zone (KLATA et al., 2000). High sodium concentration in soil and plants enhances the assimilation of magnesium in the organism, even if its content in sandy soils is low (LEONTONOWICZ et al., 1994).

In the early stages of ossification, silicon is particularly important in the areas of osseous tissue active growth. The management of this element is related to the management of calcium and magnesium. In case of calcium or magnesium deficiency, silicon enhances the process of mineralisation (BODAK et al., 1997), which may be the reason of the higher ash content in the osseous tissue of the Kent lambs.

The values of correlation coefficients for sodium and magnesium in the collected osseous tissue were the following: for Kent –  $r = 0.21$ , for Polish Merino –  $r = 0.24$ , Blackheaded –  $r = 0.25$ , for Ile de France –  $r = 0.20$ , whereas for the Leine lambs this value was the lowest ( $r = 0.03$ ) and differed from the remaining values ( $P \leq 0.05$ ). The coefficients of correlation for osseous tissue calcium and phosphorus concentrations of the studied lambs had symptomatic values, which for the Polish Merino, Blackheaded, Ile de France, and Leine were the following:  $r = 0.62$ ; ( $P \leq 0.05$ );  $r = 0.33$ ;  $r = 0.55$  ( $P \leq 0.05$ ), and  $r = 0.65$ ; ( $P \leq 0.05$ ), respectively. For the Kent lambs, the value was negative and close to zero ( $r = -0.03$ ).

The analysis of the results obtained from the studies of mineral composition of the lamb tail vertebrae osseous tissue demonstrated that the factor of the breed and the breed-related purpose type were the background of the differences between the lambs, and that the differences may have been associated to geochemical factors. Thus, these factors should also be taken into consideration in an analysis of mineral management of pregnant ewes and during the neonatal period of their offspring; their role should also be taken into account in the balanced feeding of these ruminants. Basing on the blood serum concentration of Na, K, Ca,  $P_{ig}$  and Mg in 14-day-old lambs, no conclusion should be drawn about the content of these elements in their osseous tissue.

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