THE CONTENT OF SELECTED MINERALS IN THE TIBIO-TARSAL BONE IN 14-MONTH-OLD OSTRICHES (*STRUTHIO CAMELUS*) AS INFLUENCED BY SEX AND PLACE OF THE BONE

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Summary. The content of the following mineral elements: calcium, magnesium, copper, iron, zinc and manganese collected from the specific places of the tibio-tarsal bones of healthy 14-month-old ostriches was determined for the first time.

Mineralisation was conducted in laboratory microwave system Ethos 900 by Milestone.

The research showed that concentration of a given element is dependent on the place of the studied bone. In the case of magnesium, copper and zinc, the content of the minerals was the highest in the shaft of the bone, whereas in the case of calcium, iron and manganese – in the proximal epiphysis. Interesting observations concern iron whose content was several times higher in the proximal epiphysis of the tibio-tarsal bone 9.38 (mg/kg of the fresh mass) in relation to other fragments of the bone. It seems to be connected with the high vascularisation of the proximal epiphysis located close to the pelvic bone in which active angiogenesis takes place.

Concentration of calcium in the tibio-tarsal bones of 14-month-old ostriches is statistically significantly higher in females. For the proximal epiphysis it equals 17979.12 (mg/kg of the fresh mass), for te shaft – 11548.41(mg/kg of the fresh mass), for the distal epiphysis – 16005.89 (mg/kg of the fresh mass).

The research also showed that sex is not the factor that differentiates the content of minerals in bones in a significant way (only concentration of calcium depends on sex).

The achieved results may be considered a kind of standard while determining the content of elements. Defining the values of parameters in healthy ostriches will allow to compare them with those of individuals with disorders of the bone structure.

Keywords: bones, mineral composition, ostrich.

MINERALŲ KIEKIS 14 MĖN. STRUČIŲ (*STRUTHIO CAMELUS*) BLAUZDAČIURNYJE PRIKLAUSOMAI NUO LYTIES IR KITŲ PARAMETRŲ

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Santrauka. Kalcio, magnio, fosforo, vario, geležies, cinko ir mangano kiekis tirtas 14 mėn. abiejų lyčių stručių *(Struthio camelus)* įvairiose blauzdačiurnio vietose. Mineralų kiekis tirtas mikrobangine „Ethos 900“ sistema pagal Milestone metodiką.

Nustatyta, kad mineralizacijos lygis skirtingose kaulo vietose yra nevienodas. Daugiau magnio, vario ir cinko buvo kaulų viduryje, tuo tarpu kalcio, geležies ir mangano daugiau nustatyta proksimalinėse kaulų dalyse. Ištirta, kad blauzdačiurnyje geležies buvo ženkliai daugiau (9,38 mg/kg) palyginti su kitais kaulų fragmentais. Manome, kad tai gali būti susiję su kraujagyslių išsisdėtymu ir vystymusi.

Tyrimai parodė, kad 14 mėn. stručio *(Struthio camelus)* patelių blauzdačiurnyje kalcio yra ženkliai daugiau nei patinų. Proksimaliniame epifizyje kalcio rasta 17979,12 mg/kg, kaulo viduryje – 11548,41 mg/kg, o distaliniame epifizyje – 16005,89 mg/kg šviežios kaulo masės.

Ištirta, kad paukščių lytis neturėjo įtakos kaulų mineralizacijai, išskyrus kalcio kiekį. Nustatyta, kad priklausomai nuo paukščių sveikatingumo egzistuoja tam tikri kaulų mineralizacijos lygio standartai.

Raktažodžiai: kaulai, mineralinė sudėtis, stručiai.

Introduction. Throughout the last years the development of ostrich breeding can be observed in Poland and in the world, which is also connected with the increase in the number of publications concerning technology of breeding and using ostriches (Horbańczu et al., 1998, Horbańczuk, 2000; Horbańczuk, 2002; Cooper et al.,2004; Cooper and Horbańczuk, 2004,), genetics (Horbańczuk et al., 2007; Kawka et al., 2007), feeding (Cooper et al., 2004; Cooper and Horbańczuk, 2004), reproduction (Horbańczuk et al., 1999, Malecki et al., 2005), product quality (Sales and Horbańczuk, 1998; Horbańczuk and Sales, 2001 Cooper and Horbańczuk, 2002; Horbańczuk, 2002,), behaviour (Cooper and Horbańczuk, 2002; Cooper et al., 2009), as well as body structure of these birds (Bezuienhout and Burger, 1993; Cooper and Horbańczuk, 2002; Fleming et al., 2004). However, there is very little information in scientific research concerning both locomotion as well as the way of movement of ostriches (Alexander et al., 1979; Cooper et al., 2008). The scientific research also does not have enough information on morphometrics of the limb bones of this species of birds (Charuta et al., 2007). There is also lack of data concerning the internal structure of the bone tissue and mineral composition of ostrich bones. It has to be emphasised that proper functioning of the skeleton system plays a significant role in effective breeding of these birds. Mineral components make bones and connected with organic compounds are the building material for the soft tissues of the body. It has to be stressed out that ostriches (especially the young ones) are sensitive to shortages of mineral components (Dobrowolski et al., 1998; Dorrestein, 2003). Mineralisation disorders appear mainly in young birds mainly, which show in numerous deformities of the tibio-tarsal bones. It leads to problems with moving and simultaneously to limitations in food intake and weakening of the pace of growth (Hayter, 1998). Fractures of the tibio-tarsal (*tibiotarsus*) and tarso-metatarsal (*tarsometatarsus*) bones are also common. The fractures as well as intravital deformities such as contorsions of the tibial bone (Mushi, 1999) and splayed legs can be the sign of metabolic disorders of the pelvic bones and their abnormal mineral composition. Therefore the analysis of the mineral composition of the tibio-tarsal bones of ostriches, which are the most exposed to injuries bones in the species as well as discovering the proper structure of the bone tissue and its mineral composition seem to be extremely important.

The aim of the study was to determine the content of calcium, magnesium, iron, zinc, cooper and managanese in the tibio-tarsal bones depending on sex and place of the examined bone in 14-month-old ostriches. The achieved results will also fill in the existing gap in literature data.

Materials and Methods. The analysed material consisted of the tibio-tarsal bones of ostriches taken from 17 healthy 14-month-old individuals (7 males and 10 females) fed with full-portioned pasturage for growing and bred ostriches STRUŚ PREMIUM by NUTRENA with the addition of OPTAVIT SHELL 0.3% and Calcium gluconate. The content of the studied minerals in the pasturage was compliant with feeding orders for ostriches by Horbańczuk (2002).

Before slaughtering ostriches were weighed using electronic live stock scale TP 1500/4, max load up to 15000 kg, exact to 0,5 kg. Slaughter was conducted at Zakłady Mięsne Stanisławów ltd, mazowieckie voivodship, in compliance with UN standards. The approval of Bioethical Commission was not needed as birds used for the study were routinely slaughtered. Bones for the research were stored in a freezer (the temperature below -20^{o} C). Defrosted bones were then dried at the temperature of 105^{o} C and their mass was checked at an analytic scale exact to 0.1 mg. The next stage was to extract pieces of bones and to deprive bones of their organic substance. Therefore, the bones were burnt in a microwave oven for 36 minutes. Weighed amount, 0.5-1.0 g of bones taken from specified places was prepared, the samples were always collected from the same fragments from specific bones.

The samples (fragments of bones and pasturages) were put in high-pressure Teflon-coated vessels and 5 ml of HNO_3 (Merck 1.00441) and 1 ml of H_2O_2 (Merck 1.07298) were added to them. Then the samples were mixed and left for 24 hours to be dissolved. Mineralisation was conducted in laboratory microwave system Ethos 900 by Milestone.

After mineralisation the samples were moved to calibrated flasks with a cubic content of 25 ml and filled up with deionised water (Millipore). Next the elements were marked using flame atomic absorption method with Perkin-Elmer 1100B apparatus. Metals were marked with the use of vacuum cathode lamps at suitable wavelengths.

The analysis of the results was conducted with the use of two-factor analysis of variance ANOVA using STATISTICA 6.0 programme. The studied factors were sex and type of bones. Averages were compared with Tuke's test at $p \leq 0,05$. Averages marked with the same letters do not differ statistically significantly.

Results. Tab. 1 shows the contents of particular mineral elements of the tibio-tarsal bones of 14-month-old ostriches as influenced by sex and place of the bone.

The content of calcium in the tibio-tarsal bones of 14-month-old ostriches was on average (♂♀) in the proximal epiphysis - 13563.2 mg /kg, concentration of calcium is situated evenly in particular studied fragments of the bone (Table 1). Within the species significant differences in the content of calcium were not observed depending on the place of the analysis. Looking at it referring to the content of calcium in the tibio-tarsal bone as influence by sex it was observed that females had significantly higher content of calcium. Concentration of the element in the proximal epiphysis of females was 17979.12 mg /kg , and of males - 6939.43 mg /kg – significantly lower, almost twice lower.

Another analysed element was magnesium. The research showed that concentration of magnesium in 14-month-old ostriches, similarly to copper and zinc, depended on the place of analysis and was the lowest in the shaft of the bone. The content of magnesium was on average 316 mg /kg in the shaft, in the epiphyses was significantly lower: the proximal epiphysis - 220mg /kg, and it was similar for the distal epiphysis (Table 1).

In the case of ferrum, it was shown that the proximal epiphyses of the tibial bones are characterised by statistically significantly higher kontent of the element - 9.38 mg /kg than other fragments of the studied bone (Table 1). Analysing sex groups significant statistical differences were not observed. The content of manganese in bones is spread evenly, no significant statistical differences as influenced by sex were observed. (Table 1).

Table 1. **Average content of mineral elements** (± SD) **of the tibio-tarsal bones of 14-month-old ostriches** (mg /kg of the fresh mass) **as influenced by sex and place of the bone**

	Male♂			Female♀			(♂♀)		
	Proximal epiphysis	Shaft	Distal epiphysis	Proximal epiphysis	Shaft	Distal epiphysis	Proximal epiphysis	Shaft	Distal epiphysis
Calcium (Ca)	6939.43b	9750.25b	6820.18b	17979.12a	11548.41a	16005.89a	13563.24a	10829.15a	12331.61a
±SD	1938.53	86.25	537.57	27048.49	620.85	21344.60	209.18	106.68	166.33
Magne Sium (Mg)	208.28a	328.90b	206.56a	228.81a	307.77b	214.59a	220.60a	316.22b	211.38a
±SD	61.87	19.17	19.25	72.59	18.10	18.02	65.69	20.58	17.92
Copper (Cu)	3.91a	5.00b	3.76a	4.15a	4.85b	4.08a	4.06a	4.91b	3.95a
±SD	0.61	0.11	0.43	0.57	0.49	0.15	0.56	0.47	0.15
Iron (Fe)	7.04b	1.00a	2.01a	10.94b	1.01a	1.67a	9.38b	1.01a	1.81a
±SD	0.62	0.08	0.34	4.43	0.11	0.21	3.88	0.09	0.30
Zinc (Zn)	113.00ab	154.36b	110.61a	139.77ab	155.15b	116.52a	129.07ab	154.84b	114.16a
±SD	19.71	7.46	14.63	23.59	29.80	26.96	25.09	22.63	22.01
Manganese (Mn)	1.89a	1.85a	1.67a	2.22a	2.01a	1.81a	2.09a	1.94a	1.76a
±SD	0.62	0.50	0.47	0.52	0.28	0.21	0.55	0.36	0.32

***** a,b – means with different superscript differ significantly at $p \leq 0,05$

Discussion. Proper functions of the skeletal system play extremely significant role in effective breeding of ostriches constituting the main factor limiting profitability of production, mainly of young birds whose skeleton grows intensively (between 4 and 6 month of living). At that age ostriches often experience many deformities of the tibio-tarsal and tarso-matatarsal bones which may be the result of improper feeding and poorly-balanced diet. Similarly to ostriches, illnesses of the tibio-tarsal bones also occur in other birds, for example turkeys growing intensively till they are 22 weeks old develop limb illnesses and bone formation disorders (Koncicki, 1993), (Lilburn, 1994) and fractures of long bones in older birds (Crespo et al., 2000; Tatara, 2004). Due to many anatomopathological changes of bones of ostriches, the relation of upgrowing of turkeys and its influence on mechanical resistance of the limbs bones was considered, Burs et al. (2008).

Young birds have bigger muscle tissue than bones which leads to excessive load and fractures of the pelvic bone (Korver, 2004). Intensive production of eggs requiring calcium stored in bones to make eggshells and in some cases limits to movement possibilities of females predispose to osteoporosis (Webster, 2000).

It is generally assumed that the skeleton of the vertebrates is used for storing mineral compounds, especially calcium (Sandowal, 1998).

In turn, Salicki (2006) analysed concentration of calcium in the tarsometatarsal bone of Common Wood Pigeons. The content of the element was on average 27300 mg/kg of the dry mass in adult birds.

Shortage of calcium in young birds leads to weakening, growth suppression and rachitis , Flieg (1973) and in adult birds to halisteresis and weakening of eggshells. Furthermore, calcium is essential in the process of blood coagulation. Levy et al. (1989) and Bezuidenhout (1994) occupied with establishing referential values and the content of mineral elements in blood.

Apart from calcium, magnesium is another necessary component for building bones. It is also significant in carbohydrate change. Magnesium is included in many enzymes. As commonly used food components include magnesium in sufficient amount, the shortage of it is very rare. However, in some cases the excess of it can be observed and it can lead to disorders in calcium- phosphorus balance which results in bone deformity.

The research showed that magnesium and zinc content in 14-month-old ostriches depended only on the studied place of the bone.

In the conducted analysis of the bones of ostriches high concentration of ferrum was observed in the proximal epiphysis of the bones, which is probably connected with their high vascularisation.

It seems to be connected with the high vascularisation of the proximal epiphysis located close to the pelvic bone in which active angiogenesis takes place. Kalisińska's

research (2007) showed that the concentration of iron in the tarsometatarsal bones of studied wild ducks ranged from 24.7 to 6.2 mg/kg.

As it has already been mentioned mineral components constituting the building material of the bone tissue play extremely important role in proper functioning of the whole body, therefore determining their content in healthy birds is justified. Bezuidenhout (1994) worked on determining mineral composition of the tibio-tarsal bone in 4 and 8-week-old healthy and ill ostriches, which had bone rotation. However, the research of that type was not conducted in adult ostriches. Bezuidenhout's study (1994) showed that the content of calcium in young healthy ostriches was 884.8 mg/kg , whereas ill ostriches had 803.60 mg/kg of it.

Summing up, analysis of the content of particular mineral elements showed that significant difference between males and females of ostriches in the content of elements is observed in the case of calcium concentration, more calcium is observed in bones of females. The concentration of ferrum and manganese i salso higher in females, however, no significant statistical differences were observed. In the case of magnesium, copper and zinc, the content of the minerals was the highest in the shaft of the bone, whereas in the case of calcium, ferrum and manganese – in the proximal epiphysis.

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