Animal Science Papers and Reports vol. 33 (2015) no. 4, 383-398
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Bone morphometric, densitometric and mechanical properties in 14-month-old ostriches fed experimental diet enriched with linseed*

Anna Charuta[1], Marcin Tatara[2, 3], Witold Krupski[3], Ewa Poławska[4], Magdalena Ogłuszka[4], Mariusz Pierzchała[4], Iwona Łuszczewska-Sierakowska[5]**

[1] Vertebrates Morphology Department, Department of Zoology, Institute of Biology, Faculty of Natural Science, Siedlce University of Natural Sciences and Humanities, 08-110 Siedlce, Poland

[2] Department of Animal Physiology, Faculty of Veterinary Medicine, University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland

[3] II Department of Radiology, Medical University in Lublin, Staszica 16, 20-081 Lublin, Poland

[4] Institute of Genetics and Animal Breeding PAS, 05-552 Jastrzębiec, Poland

[5] Chair of Human Anatomy, Department of Normal Anatomy, Medical University in Lublin, Jaczewskiego 4, 20-090 Lublin, Poland

(Accepted October 21, 2015)

The aim of the study was to evaluate the influence of the diet enriched with linseed on morphometric, densitometric and mechanical properties of tibio-tarsus and tarso-metatarsus in 14-month-old ostriches.

The experimental diet including 4% of linseed was applied to ostriches (N=8) starting from the attainment of 45 kg of body weight, while the control birds (N=8) were fed the standard diet. At the age of 14 months of life, blood samples were collected and the birds were slaughtered to isolate left tibio-tarsus and tarso-metatarsus.

---

*This study was partially financed within the project "BIOFOOD" (innovative, functional products of animal origin) No. POIG.01.01.02-014-090/09, which was co-financed by the European Regional Development Fund and partially financed within the subject of the statutory (244/08/S-Siedlce University of Natural Sciences and Humanities).

**Corresponding author: anna.charuta@neostrada.pl; anna.charuta@uph.edu.pl

Dual-energy X-ray absorptiometry method was used to evaluate the bone mineral density and bone mineral content. Using quantitative computed tomography, total bone volume, mean volumetric bone mineral density, volumetric bone mineral density of trabecular and cortical bone, cortical bone area, calcium hydroxyapatite density of the trabecular and cortical bone were also determined. Cross-sectional area, second moment of inertia, mean relative wall thickness and cortical index were determined. Maximum elastic strength and ultimate strength of bones were determined using three-point bending test. Total antioxidative capacity in the serum was measured using commercial photometric test. In the experimental group of males, cortical bone area reached significantly higher value, while calcium hydroxyapatite density of the trabecular bone of tibio-tarsus was significantly lower, when compared to sex-matched controls (P<0.05).

In conclusion, the experimental diet enriched with 4% of linseed, starting from 45 of kg of body weight of birds, had neither the positive nor negative effects on the evaluated bone properties in 14-month-old ostriches. Thus, higher dietary dosage of linseed or different administration period of the experimental diet should be applied to expect effects on skeletal system quality in growing ostriches.

KEY WORDS: linseed / ostrich / skeletal system quality / quantitative computed tomography / tibio-tarsus / tarso-metatarsus

Increased interest in Ratitae breeding, including ostriches (*Struthio camelus var. domesticus*) is observed recently [Cooper and Horbańczuk 2004, Kawka *et al*. 2007, 2012a, b, Rybnik *et al*. 2007, Poławska *et al*. 2011]. Ostriches are considered as an alternative poultry species providing very high quality meat and skins [Hoffman *et al*. 2008, Sales and Horbańczuk 1998, Sales *et al*. 1999, Horbańczuk *et al*. 1998, 2007, 2008, Poławska *et al*. 2011, 2013]. It may be predicted that in Poland and in whole Europe the development of breeding farms will be observed in nearest future. However, intensive growth rate observed in ostriches breeding at farm conditions is associated with increased occurrence of development-origin bone disorders, especially concerning long bones in pelvic limb and affecting locomotory functions of the birds [Horbańczuk *et al*. 2004, Cooper 2007, Cooper *et al*. 2008]. Thus, modification of the standard diet applied to ostriches during breeding period is recommended to improve skeletal system quality and diminish bone disorders occurrence. Bone-related disorders affects negatively economic outcomes of ostriches breeding process, the animal welfare, the health status and the final meat product quality. Thus, novel effective breeding strategies based on dietary factors and leading to improvement of ostrich skeletal system quality, meat quality, animal welfare and health status of birds should be evaluated and introduced.

Omega-3 (O-3) and omega-6 (O-6) fatty acids (FAs) and their common ratio (O-6FAs/O-3FAs) were recently investigated for better understanding their potential effects on skeletal system [Baird *et al*. 2008, Ebeid 2011, Ebeid 2011a; Patwardhan *et al*. 2011, Faitarone *et al*. 2012, Hosseini-Vashan *et al*. 2014]. To improve meat product quality in poultry species, dietary administration with oilseeds is applied [Poławska *et al*. 2012]. Thus, the inclusion of plant oils to the ostrich diet seems to be interesting and important for meat industry and consumers. Supplements added to the ostrich diet may affect significantly both technological parameters and sensory properties of ostrich meat, as well as skeletal system quality. Effects of dietary inclusion of oil plant seeds in ostriches on their skeletal system quality were not reported previously.

Thus, the aim of the study was to evaluate the effect of the experimental diet enriched in 4% of linseed on serum total antioxidative capacity and morphometric, densitometric and mechanical properties of tibiotarsus and tarsometatarsus in 14-month-old ostriches.

Material and methods

The experimental procedures used in this study were approved by The 3rd Local Ethics Committee on Animal Experimentation in Warsaw (SGGW Warsaw) in a Resolution No 27/2009.

Experimental design

All control and experimental birds (*Struthio camelus var. domesticus*) were kept together from hatching to 14 months of age in standard breeding conditions on a commercial ostrich farm in Stypułów (western Poland). The farm is under scientific supervision of the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences. All birds were fed standard starter diet until 45 kg of body weight (BW) and then assigned to two groups: control group (N=8), where birds were fed all the time standard diet, divided into female (N=4) and male (N=4) subgroups, and experimental group, where birds were fed experimental diet enriched with 4% of linseed, divided into female (N=4) and male (N=4) subgroups. The experimental diet was prepared on the base of the control diet, a part of which was replaced with 4% of linseed. Protein and gross energy contents (150 g kg^{-1} crude protein and 2550 kcal kg^{-1} gross energy) were kept constant across the study (Tab. 1) [Poławska *et al.* 2012].

Table 1. Composition of the control and experimental diets applied to ostriches from 45 kg of body weight [Poławska *et al.* 2012]

Ingredient (g/kg)	Control diet	Experimental diet (enriched with 4% of linseed)
Barley	330	340
Wheat (12% CP)	355	290
Wheat bran	160	190
Soybean meal (45.5% CP)	105	90
Linseed	-	40
Vitamin mineral premix	50	50
Energy and nutrient content		
Dry matter	885	886
Crude protein	151	151
Crude fat	21.5	39.4
Crude fiber	45.3	50.4
Ash	68	68
Starch	414	386
Energy (MJ/kg)	11	11

All birds were slaughtered in commercial abattoir in Wolbrom (Poland) at 14 month of age when their mean live weight had reached 94.53±1.98 kg. Ostriches were fasted for 24 h before slaughter procedure. Electrical stunning, bleeding and evisceration were performed according to the standard slaughtering procedures for ostriches [Majewska *et al.* 2009]. Blood samples for serum were collected during bleeding procedure.

Immediately after the slaughter of ostriches, left tibiotarsus and tarsometatarsus were isolated, cleaned from soft tissues and the bone weight and length were determined. Bone samples (placed in plastic bags) and serum samples (frozen in liquid nitrogen) were transported from Wolbrom to University of Life Sciences in Lublin for storage at -25°C until further analyses.

Dual energy X-ray absorpciometry

Whole bones isolated from ostriches were thawed in bags for 3 hours at room temperature and scanned using dual energy X-ray absorpciometry (DEXA) method to determine the bone mineral density (BMD) and bone mineral content (BMC). Scanning procedure was performed using Norland XR-46 densitometer (resolution 3.0 x 3. mm) and Research Scan software (Norland, Fort Atkinson, USA). All bones were placed on dorsal surface and scanned in anterior-posterior direction. Results of BMD and BMC measurements were expressed in g/cm^2 and g, respectively.

Quantitative computed tomography

Volumetric bone mineral density (vBMD) of the trabecular bone (Td – trabecular bone mineral density) and cortical bone (Cd – cortical bone mineral density) of the tibiotarsus and tarsometatarsus were determined in g/cm^3 using quantitative computed tomography (QCT) method and Somatom Emotion Siemens apparatus supplied with Somaris/5 VB10B software (Siemens, Erlangen, Germany). Thawed bones were scanned using 2-mm thick cross-sectional sequential scans. The measurement of vBMD was performed for the trabecular and cortical bone on cross-sectional metaphyseal and diaphyseal 2-mm thick QCT scans. Trabecular bone mineral density of the tibiotarsus was determined in the distal epiphysis of bone at 5 % of total bone length, measuring from the distal extremity, just below growth plate. Trabecular bone mineral density of the tersometatarsus was determined in the proximal epiphysis of bone at 3 % of total bone length, measuring from the proximal extremity, just above visible growth plate. Cortical bone mineral density was measured on mid-diaphyseal scan placed at 50% of the tibiotarsus and tarsometatarsus length. Cortical bone area (CBA) was measured automatically at the midshaft of the bones. Calcium hydroxyapatite density of the trabecular bone ($Tb_{Ca\text{-}HA}$) and calcium hydroxyapatite density of the cortical bone ($Cb_{Ca\text{-}HA}$) were determined using 10-mm thick epiphyseal and middiaphyseal scans (as described above) and Osteo CT application package. To determine $Tb_{Ca\text{-}HA}$ and $Cb_{Ca\text{-}HA}$, the water- and bone-equivalent calibration phantom was used as the reference standard. Volume evaluation software (Siemens, Erlangen, Germany) was used to determine the

total bone volume (Bvol) and mean volumetric bone mineral density (MvBMD) of each tibiotarsus and tarsometatarsus. For Bvol and MvBMD measurements, the volume-of-interest was limited by a minimum and maximum density of the investigated bones at 0 and 3000 Hounsfield units, respectively. The measurements of Bvol and MvBMD were executed for the whole bones, and obtained results reflect the values determined within all anatomic structures of the investigated bones.

Determination of geometric properties of tarsometatarsus

Geometrical properties of each tibiotarsus and tersometatarsus were determined on the basis of measurements of horizontal and vertical diameters (both external and internal) of the mid-diaphyseal cross-section of the bone obtained from computed tomography multiplanar reconstructions. The values of cross-sectional area (A), second moment of inertia (Ix), mean relative wall thickness (MRWT) and cortical index (CI) were calculated [Brodzki *et al*. 2004, Tatara *et al.* 2005b, Tatara *et al.* 2007].

Three-point bending test

Mechanical properties of the tibiotarsus and tersometatarsus were determined using the three-point bending test in Instron 3367 apparatus (Instron, Canton, MA, USA). The relationship between loading force perpendicular to the longitudinal axis of the bone and the resulting displacement was presented graphically. The values of maximum elastic strength (Wy) and the ultimate strength (Wf) were determined. The distance between supports of the bone was set at 40% of total tibiotarsus and tarsometatarsus length and the measuring head loaded bone samples at the midshaft with a constant speed of 50 mm/min [Tatara *et al.* 2007].

Determination of total antioxidative capacity in serum

Total antioxidative capacity (TAC) determination in serum collected from fasted ostriches was performed using commercial photometric test system (ImAnOx (TAS) Kit, Immundiagnostik AG, Bensheim, Germany). The photometric measurement was performed with the use of Benchmark Plus microplate spectrophotometer supplied with Microplate Manager Software Version 5.2.1 (Bio-Rad Laboratories Inc., Hercules, CA, USA) and TAC values were expressed in μmol/L. As indicated by the TAS Kit supplier, the values below 280 μmol/L were considered as low TAC, while the values between 280 – 320 μmol/L were considered as middle level of TAC. High TAC was recognized when the values exceed 320 μmol/L [Brodzki *et al*. 2015].

Statistical analysis

All data are presented as means±SEM. Differences between means were tested for statistical significance with the use of non-paired Student's *t*-test for non-dependent variables. Statistical comparison was performed between experimental and control groups, as well as between experimental and control subgroups of males and females. For each comparisons, the difference showing a P-value <0.05 was considered as statistically significant.

Results and discussion

The final body weight determined just before transportation of ostriches to slaughterhouse were not significantly (P=0.12) different between the experimental (97.4±2.7 kg) and control group (91.3±2.5 kg). Body weight in the subgroups of the control males and females reached 89.0±1.7 kg and 93.0±4.3 kg, while in the experimental subgroups these values were 96.7±4.3 kg and 97.8±3.9 kg, respectively. The differences of body weight between the sex-matched experimental and control subgroups were not statistically significant (P>0.05). Total antioxidative capacity of the serum obtained from 14-months old ostriches was not different between the experimental (252.7±39.6 µmol/l) and control group (291.0±9.9 µmol/l; P=0.44). Total antioxidative capacity in the subgroups of control males and females reached 277.8±18.9 µmol/l and 301.0±9.4 µmol/l, while in the experimental subgroups these values were 280.1±25.9 µmol/l and 239.0±62.4 µmol/l, respectively. The differences of TAC between the sex-matched experimental and control subgroups were not statistically significant (P>0.05). The values of the evaluated morphological, densitometric, and mechanical parameters of tibiotarsus and tarsometatarsus in the control and experimental groups are shown in Table 2 and 4. Cortical bone area of tibiotarsus shown clear tendency to be higher in the experimental group when compared to this value in the control group (P=0.07). All the other evaluated parameters of tibiotarsus and tarsometatarsus were not significantly different between the experimental and control group (P>0.05). The

Table 2. Morphological, densitometric and mechanical parameters of tibiotarsus in the control and experimental group of 14-month-old ostriches

Investigated parameter	Control group	Experimental group	P-value
Bone length (mm)	540±5	532±7	0.41
Bone weight (g)	1039±32	1078±29	0.38
Relative bone weight	0.01±0.00	0.01±0.00	0.27
Bone mineral density (g/cm^2)	1.76±0.07	1.83±0.03	0.35
Bone mineral content (g)	367±15	385±9	0.34
Cortical bone area (mm^2)	456±20	505±15	0.08
Total bone volume (cm^3)	578±21	614±14	0.18
Mean volumetric bone mineral density (g/cm^3)	1.80±0.02	1.79±0.02	0.65
Trabecular bone mineral density (g/cm^3)	1.40±0.01	1.39±0.02	0.50
Cortical bone mineral density (g/cm^3)	2.59±0.03	2.57±0.04	0.73
Calcium hydroxyapatite density of the trabecular bone (mg/ml)	261±10	237±15	0.19
Calcium hydroxyapatite density of the cortical bone (mg/ml)	1223±16	1229±26	0.84
Cross-sectional area (mm^2)	501±28	502±16	0.97
Second moment of inertia (mm^4)	39002±2712	35673±2378	0.37
Mean relative wall thickness	0.63±0.07	0.64±0.03	0.79
Cortical index	37.69±2.63	38.98±1.09	0.66
Maximum elastic strength (N)	7429±680	7758±359	0.68
Ultimate strength (N)	9732±696	10206±318	0.55

values of the morphological, densitometric, and mechanical parameters of tibiotarsus and tarsometatarsus in the control and experimental subgroups of males and females are shown in Table 3 and 5. Cortical bone area of tibia reached significantly higher value in the subgroup of experimental males, when compared to the subgroup of

Table 3. Morphological, densitometric and mechanical parameters of tibiotarsus in the male and female subgroups of the control and experimental groups of 14-month-old ostriches

Investigated parameter	Control group		Experimental group	
	males	females	males	females
Bone length (mm)	538±11	541±5	516±15	542±4
Bone weight (g)	1037±26	1040±56	1094±47	1068±40
Relative bone weight	0.02±0.00	0.01±0.00	0.01±0.00	0.01±0.00
Bone mineral density (g/cm^2)	1.82±0.06	1.71±0.11	1.82±0.04	1.84±0.04
Bone mineral content (g)	374±8	361±28	385±15	384±13
Cortical bone area (mm^2)	484^a±12	436±32	533^b±11	488±21
Total bone volume (cm^3)	582±27	575±34	620±9	611±23
Mean volumetric bone mineral density (g/cm^3)	1.79±0.03	1.80±0.02	1.77±0.03	1.80±0.02
Trabecular bone mineral density (g/cm^3)	1.40±0.03	1.41±0.02	1.33±0.01	1.42±0.02
Cortical bone mineral density (g/cm^3)	2.56±0.01	2.61±0.06	2.50±0.06	2.63±0.03
Calcium hydroxyapatite density of the trabecular bone (mg/ml)	265^a±17	258±13	200^b±16	259±14
Calcium hydroxyapatite density of the cortical bone (mg/ml)	1197±12	1242±24	1194±37	1250±34
Cross-sectional area (mm^2)	535±42	475±36	502±28	502±22
Second moment of inertia (mm^4)	41142±5021	37397±3336	35502±3643	35776±3441
Mean relative wall thickness	0.71±0.10	0.56±0.09	0.65±0.05	0.64±0.04
Cortical index	40.97±3.73	35.24±3.56	39.18±1.74	38.86±1.56
Maximum elastic strength (N)	7433±737	7425±1161	7817±758	7722±433
Ultimate strength (N)	9962±169	9560±1287	10490±621	10035±385

[ab]Means bearing different superscripts differ significantly at P≤005.

Table 4. Morphological, densitometric and mechanical parameters of tarsometatarsus in the control and experimental group of 14-month-old ostriches

Investigated parameter	Control group	Experimental group	P-value
Bone length (mm)	470±5	461±6	0.301
Bone weight (g)	616±17	626±20	0.72
Relative bone weight	0.07±0.00	0.01±0.00	0.14
Bone mineral density (g/cm^2)	1.41±0.04	1.46±0.02	0.31
Bone mineral content (g)	241±9	251±6	0.34
Cortical bone area (mm^2)	392±18	400±10	0.74
Total bone volume (cm^3)	353±12	356±10	0.87
Mean volumetric bone mineral density (g/cm^3)	1.93±0.03	1.91±0.02	0.61
Trabecular bone mineral density (g/cm^3)	1.40±0.02	1.42±0.02	0.47
Cortical bone mineral density (g/cm^3)	2.52±0.03	2.48±0.03	0.37
Calcium hydroxyapatite density of the trabecular bone (mg/ml)	303±20	290±12	0.59
Calcium hydroxyapatite density of the cortical bone (mg/ml)	1129±19	1086±33	0.28
Cross-sectional area (mm^2)	387±11	405±11	0.25
Second moment of inertia (mm^4)	25973±1157	27637±1047	0.31
Mean relative wall thickness	0.69±0.05	0.72±0.05	0.70
Cortical index	40.48±1.65	41.44±1.77	0.70
Maximum elastic strength (N)	6343±251	6700±240	0.32
Ultimata strength (N)	8076±456	8371±333	0.61

control males (P =0.03). Significantly lower value of $Tb_{Ca\text{-}HA}$ of tibiotarsus was reached in the subgroup of experimental males when compared to the subgroup of control males (P=0.05). Cross-sectional area of tarsometatarsus of the experimental subgroup males has shown tendency to be higher when compared to the subgroup of control males (P=0.07). Similar tendencies in such comparison were shown for MRWT and CI (P=0.08 and P=0.07).

The current study was undertaken to explain whether the experimental diet enriched with 4% of linseed affects morphological, densitometric and mechanical properties of tibiotarsus and tarsometatarsus in growing ostriches. It was hypothesized that linseed inclusion to the experimental diet may improve skeletal system quality in ostriches. Both tibiotarsus and tarsometatarsus play important supportive functions in bipedal ostriches and may serve as experimental models for determination of skeletal system quality in different meat-type poultry species [Charuta *et al*. 2008, Charuta *et al*. 2010, Dzierzęcka and Charuta 2010, Barreiro *et al*. 2011, Charuta *et al*. 2011, Charuta 2012, Ahmed *et al*. 2013; Charuta *et al*. 2013, Charuta *et al*. 2013a, Charuta *et al*. 2013, Charuta *et al.* 2013a, Charuta *et al*. 2014].

Proper structure of several bones and mineral content in bones of the pelvic limb bones is crucial for the optimal health status, locomotory functions and the growth rate in current poultry breeding practice of meat-type poultry focused on shortening breeding course [Biesiada-Drzazga 2014]. Delayed skeletal system mineralization process in comparison to the formation of skeletal muscles and rapid muscle tissue

Table 5. Morphological, densitometric and mechanical parameters of tarsometatarsus in the male and female subgroups of the control and experimental groups of 14-month-old ostriches

Investigated parameter	Control group		Experimental group	
	males	females	males	females
Bone length (mm)	466±8	473±8	471±11	455±6
Bone weight (g)	612±21	619±29	645±36	614±25
Relative bone weight	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
Bone mineral density (g/cm^2)	1.43±0.04	1.41±0.08	1.50±0.00	1.44±0.02
Bone mineral content (g)	238±11	242±15	264±8	244±7
Cortical bone area (mm^2)	399±32	387±25	429±6	382±7
Total bone volume (cm^3)	337±11	365±17	361±16	353±14
Mean volumetric bone mineral density (g/cm^3)	1.91±0.04	1.94±0.04	1.92±0.04	1.91±0.02
Trabecular bone mineral density (g/cm^3)	1.40±0.04	1.40±0.03	1.44±0.02	1.41±0.02
Cortical bone mineral density (g/cm^3)	2.46±0.05	2.6±0.03	2.45±0.06	2.50±0.04
Calcium hydroxyapatite density of the trabecular bone (mg/ml)	278±38	322±21	314±24	276±9
Calcium hydroxyapatite density of the cortical bone (mg/ml)	1110±27	1144±27	1060±52	1101±46
Cross-sectional area (mm^2)	399±16	378±15	428±7	392±14
Second moment of inertia (mm^4)	27022±939	25187±1940	28394±1963	27184±1332
Mean relative wall thickness	0.75±0.10	0.65±0.05	0.84±0.08	0.65±0.05
Cortical index	42.36±3.26	39.08±1.66	45.13±2.34	39.23±1.97
Maximum elastic strength (N)	6167±338	6475±388	6817±342	6630±351
Ultimate strength (N)	8132±879	8033±583	8819±548	8102±415

gain in meat-type poultry species leads to the frequent occurrence of deformations, rotations and fractures of long bones in the pelvic limbs [Horbańczuk *et al*. 2004, Cooper 2007, Cooper and Horbańczuk 2004, Tykałowski *et al.* 2010, Charuta *et al.* 2011, Charuta *et al*. 2012]. Considering huge skeletal muscle tissue gain in meat-type poultry species and resulting excessive skeletal system loading, diagnostic monitoring

of skeletal system quality during several phases of posthutching development is recommended [Charuta *et al.* 2012, Charuta *et al.* 2013, Charuta *et al.* 2014]. To improve skeletal system quality and mechanical endurance of bones in the growing birds, an inclusion of feed additives and oil plants to the diet influencing bone tissue metabolism are also recommended [Tatara *et al.* 2003, Tatara *et al.* 2006, Baird *et al.* 2008, Janocha *et al.* 2009, Tatara *et al.* 2009, Cheng *et al.* 2011, Ebeid 2011, Patwardhan *et al.* 2011, Faitarone *et al.* 2012, Hosseini-Vashan *et al.* 2014]. However, to obtain optimal effectiveness of the nutritional factors on bone tissue metabolism the determination of the most advantageous feeding protocols is required.

Effects of dietary inclusion of oil plants on skeletal system properties were evaluated in numerous previous studies. In the experiment performed by Puzio [2012], the determination of the effect of dietary inclusion of linoleic acid and false flax (*Camelina sativa*) on skeletal system properties in broiler chickens was performed. It was shown that false flax oil and linoleic acid may replace effectively sunflower oil in the diet of broiler chicken. False flax oil affected positively BMD and BMC of femur, tibia and humerus in broiler chickens. Bone weight, BMD and BMC of the investigated bones were significantly increased in the group of broilers receiving both false flax oil and linoleic acid when compared to the group fed with lioleic acid solely. Moreover, BMC of the investigated bones was significantly higher in birds receiving solely linoleic acid and fals flax oil when compared to the controls. Studies on Japanese quails by Ebeida [2001a] have shown that dietary administration of the fish oil and linseed oil has beneficial effects on morphological parameters and mechanical endurance of tibia. Weight, length, diaphysis diameter, breaking strength and ash content in tibia were found to be significantly higher in birds receiving fish oil and linseed when compared to control group [Ebeida 2001a]. However, in contrast to the dietary administration of linseed, fish oil in the diet may affects negatively sensory traits of meat such as fishy odor and flavor limiting its application in meat-type poultry nutrition. O-3 polyunsaturated fatty acid (PUFA) lipid autoxidation process is considered as the main cause of fishy odor and flavor [Ganesan et al. 2014]. In other studies performed by Ebeida [2001b] on laying hens fed the diet enriched with PUFA from fish and linseed oils, negative effects of the experimental diet on structural properties of tibiotarsus were not stated. Experiments performed by Faitarone [2012] have shown that dietary administration of O-3 and O-6 PUFA to laying hens has not influenced the formation and resorption of bone tissue of tibia. The inclusion of vegetable oils in the diet of laying hens has leaded to reduced bone mineral retention, however this reduction was mitigated when supplementation with highest inclusions of oils rich in O-3 was performed [Faitarone 2012]. Positive effects of feeding with linseed and palm oils on growth performance and bone tissue metabolism in broiler chickens were reported by Zong [2014]. In the study on broiler chickens, no significant differences in weight gain, feed intake and gain/feed ratio were observed between birds fed with the lard and linseed oil. Broilers fed palm oil had significantly higher weight gain and feed intake than those fed lard or linseed oil. Growth performance in palm oil group and in linseed oil + palm oil

group was significantly greater than that of single-oil groups. Tibia growth and bone characteristics were not influenced by supplementation with the lard, linseed oil and palm oil alone, but broilers fed the mixture of fats were characterized by significantly higher tibia weight and length, when compared to broilers fed on linseed oil. Bone mineral density of tibia was significantly increased in palm oil group and linseed oil + palm oil groups when compared to single-oil groups. Dietary administration of the linseed oil alone or in the combination with palm oil enhanced apparent digestibility of calcium, reduced serum calcium concentration and increased tibia calcium content. Moreover, supplementation with the linseed oil alone or in the combination with palm oil had a positive effect on biochemical markers of bone growth. The combination of the linseed and palm oils was beneficial for growth performance, tibia growth and biomarkers of bone metabolism [Zong 2014]. Experiments on White Leghorn chickens have shown no effects of dietary inclusion of O-3 and O-6 fatty acids on BMD of tibia and humerus [Mazzuco *et al*. 2005]. Mazzuco *et al*. [2005] has applied an O-6 to O-3 ratio of 0.6:1 or 8:1 in the diets of chickens and has found no effect in BMD between the diets. In other studies [Bairda 2008], various ratios of O-6 to O-3 fatty acids in the diets of mature White Leghorn chickens have not affected bone characteristics or bone strength parameters, except for cortical thickness which was significantly increased as O-3 in the diet has been increasing until the highest level of O-3 in the diet. However, an increased cortical thickness has not affected bone strength. Thus, it was suggested to determine in further studies potential effects of dietary inclusion of O-3 fatty acids on skeletal system quality in growing birds. Studies by Johnston *et al*. [2006] have demonstrated similar findings in turkeys. The BMD and BMC of turkey hens were not significantly different between 2 extreme diet treatments, one rich in O-3 fatty acids (O-6 to O-3 ratio of 1.7:1) and the other rich in O-6 fatty acids (O-6 to O-3 ratio of 19:1) [Johnston *et al.* 2006]. Liu *et al*. [2003] have found significantly higher BMC in quails fed a fish oil-supplemented diet (rich in O-3) when compared to the group fed with a soybean oil diet (rich in O-6). The previous study in the mature quails has demonstrated that long-term supplementation of lipids in the diets altered fatty acid composition of bone lipids, which reflects the fatty acid profile of the diet. In studies with rats, no significant difference in bone area, BMD and BMC were found between the groups fed diets rich in O-3 fatty acids when compared to the diets rich in O-6 fatty acids [Mollard *et al*. 2005]. In the other study on rodents, fish oil-supplemented rats (rich O-3 fatty acids diet) had significantly higher BMD in the distal femur and proximal tibia than a corn oil-supplemented animals (rich O-6 fatty acids diet) [Bhattacharya *et al.* 2005].

The results obtained in the current study have shown relatively poor effects of the experimental diet enriched with 4% of linseed on the investigated morphometric, densitometric and mechanical properties of tibiotarsus and tarsometatarsus. The application of the experimental diet in the subgroups of male ostriches increased only CBA and decreased $Tb_{Ca\text{-}HA}$ of tibiotarsus. Similar clear tendency to increased CBA value of tibiotarsus was found comparing whole experimental group of birds with the

control group. Based on the observed relatively poor effectiveness of the experimental diet on the bone properties in this study, it may be postulated to change the dosage of linseed in the diet to obtain improved skeletal system quality. Besides linseed dosage manipulation, its inclusion to the diet at different systemic growth stages of ostriches may provide possibly more spectacular effects on bone tissue metabolism regulation. It may be postulated that 45 kg of body weight ostriches are in relatively advanced stage of systemic and skeletal growth and development, and earlier inclusion of linseed to the diet may have more visible effects. It is worth to underline that BMD, BMC, most of the morphometric/geometric parameters and mechanical endurance indices of tibiotarsus and tarsometatarsus reached numerically higher values in the experimental ostriches; however, due to relatively high costs of the experiment and limited number of animals in the groups, beneficial effects of the experimental diet on skeletal system quality was not confirmed statistically. Thus, higher number of animals engaged to the experiment may be recommended for further studies.

In conclusion, this study has shown that the experimental diet enriched with 4% of linseed, applied to the ostrich diet from 45 of kg of body weight of birds, had neither the positive nor negative effects on the evaluated bone properties in 14-month-old ostriches. Thus, higher dietary dosage of linseed or different administration period of the experimental diet should be applied to expect more visible effects on skeletal system quality in growing ostriches.

REFERENCES

1. AHMED Y. A., SOLIMAN S. A., ABDEL-HAFEZ E. A., 2013 – Ossification of the femur and tibia of the post-hatching Japanese quail. ***Pakistan Journal of Biological Sciences*** 16, 859-64.
2. BAIRD H.T., EGGETT D.L., FULLMER, S., 2008 – Varying ratios of omega-6:omega-3 fatty acids on the pre- and postmortem bone mineral density, bone ash, and bone breaking strength of laying chickens. ***Poultry Science*** **87**, 323-328.
3. BARREIRO F.R., BARALDI-ARTONI S.M., DO AMARAL L.A, BARBOSA J.C., GIRARDI A.M., PACHECO M.R, AMOROSO L., 2011 – Determination of broiler femur parameters at different growth phases. ***International Journal of Poultry Science*** 10, 849-853.
4. BHATTACHARYA A., RAHMAN M., BANU J., LAWRENCE R.A., MCGUFF H.S., GARRETT I.R., FISCHBACH M., FERNANDES G., 2005 – Inhibition of osteoporosis in autoimmune disease prone MRL/Mpj-Fas(lpr) mice by N-3 fatty acids. ***The Journal of the American College of Nutrition*** 24, 200-209.
5. BIESIADA-DRZAZGA B., 2014 – Growth and slaughter value of W_{11}, W_{33} and W_{31} White Kołuda geese. ***European Poultry Science*** 78. ISSN 1612-9199.
6. BRODZKI A., BRODZKI P., TATARA M.R., KOSTRO K., 2015 – Total antioxidative capacity and zinc concentration in dogs suffering from perianal tumours. ***Bulletin of the Veterinary Institute in Pulawy*** 59, 417-423.
7. BRODZKI A., SILMANOWICZ P., TATARA M.R., BRODZKI P., ORZELSKI M., 2004 – Cortical index (CI) of dogs' femur assessment in determination of mechanical and geometrical properties of skeletal system. ***Medycyna Weterynaryjna*** 60, 1056-1060.
8. CHARUTA A., 2013 – Evaluation of densitometric and geometric parameters of the femur in 14-month-old ostriches depending on sex with the use of computed tomography. ***Bulletin of the Veterinary Institute in Pulawy*** 57, 287-291.

9. CHARUTA A., COOPER R. G., PIERZCHAŁA M., HORBAŃCZUK J.O., 2012 – Computed tomographic analysis of tibiotarsal bone mineral density and content in turkeys as influenced by age and sex. ***Czech Journal of Animal Science*** 57, 572-578.
10. CHARUTA A., DZIERZĘCKA M., COOPER R.G., 2010 –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. ***Veterinarija ir Zootechnika*** 52, 8-12.
11. CHARUTA A., DZIERZĘCKA M., KOMOSA M., BIESIADA-DRZAZGA B., DZIAŁA-SZCZEPAŃCZYK E., COOPER R. G., 2013 – Age-and sex-related changes in mineral den sity and mineral content of the tibiotarsal bone in quails during post-hatching development. ***Kafkas Üniversitesi Veteriner Fakültesi Dergisi Journal*** 19, 31-36.
12. CHARUTA A., DZIERZĘCKA M., KOMOSA M., KALINOWSKI Ł., PIERZCHAŁA M., 2013 – Age- and sex- related differences of morphometric, densitometric and geometricparameters oftibiotarsal bone in Ross broiler chickens. ***Folia Biologica (Kraków)*** 61, 211-220.
13. CHARUTA A., DZIERZĘCKA M., MAJCHRZAK T., CZERWIŃSKI E., COOPER R.G., 2011 – Computer-generated radiological imagery of the structure of the spongious substance in the postnatal development of the tibiotarsal bones of the Peking domestic duck (*Anas platyrhynchos var. domestica*). ***Poultry Science*** 90, 830-835.
14. CHARUTA A., DZIERZĘCK M., PIERZCHAŁA M., COOPER R. G., POŁAWSKA E., HORBAŃCZUK J.O., 2013a – Sex-related differences of morphometric. densitometric and geometric parameters of tibia and tarsometatarsal bone in 14-month-old ostriches (*Struthio camelus*). ***Poultry Science*** 92, 2965-76.
15. CHARUTA A., MAJCHRZAK T., CZERWIŃSKI E., COOPER R.G., 2008 – Spongious matrix of the tibiotarsal bone of ostriches (*Struthio camelus*) a a digital analysis. ***Bulletin of the Veterinary Institute in Pulawy*** 52, 175-178.
16. CHARUTA A., TATARA M.R., GRUŻEWSK A A., PIERZCHAŁA M,. KALINOWSKI Ł, TRUSEWICZ M., ŁUSZCZEWSKA-SIERAKOWSKA I., 2014 – Morphological and densitometric research of the tibial bone in the post-natal development in domestic geese. ***Animal Science Papers and Reports*** 32, 251-260.
17. CHENG J., PENG K., JIN E., ZHANG Y., LIU Y., ZHANG N., SONG H., LIU H., TANG Z., 2011- Effect of additional boron on tibias of African ostrich chicks. ***Biological Trace Element Research*** 144, 538-49.
18. COOPER R.G., HORBAŃCZUK J.O., 2004 – Ostrich nutrition: a review from a Zimbabwean perspective. Monography. ***Revue Scientifique et Technique de L Office International Des Epizooites*** 23, 1033-1042.
19. COOPER R. G., NARANOWICZ H., MALISZEWSKA E., TENNET A., HORBAŃCZUK J.O., 2008 – Sex-based comparison of limb segmentation in ostriches aged 14 months with and without tibiotarsal rotation. ***Journal of the South African Veterinary Association*** 79, 142-1144.
20. COOPER R.G., TOMASIK C., HORBAŃCZUK J.O., 2007 – Avian Influenza in Ostriches (*Struthio camelus*). ***Avian and Poultry Biology Reviews*** 18, 87-92.
21. DZIERZĘCKA M., CHARUTA A., 2010 – Influence of sex on bone mineral density and content of the skeleton of the thoracic and pelvic limb in the ostrich (*Struthio camelus var. domesticus*). ***Bulletin of the Veterinary Institute in Pulawy*** 54,601-604.
22. EBEID- T. A., 2011 – The impact of incorporation of n-3 fatty acids into eggs on ovarian follicular development, immune response, antioxidative status and tibial bone characteristics in aged laying hens. ***Animal*** 10, 1554-1562.
23. EBEID T., FAYOUD A., EL-SOUD S. A., EID Y., EL-HABBAK M., 2011 – The effect of omega-3 enriched meat production on lipid peroxidation, antioxidative status, immune response and tibia bone characteristics in Japanese quail. ***Czech Journal of Animal Science*** 56, 314-324.

24. FAITARONE A.B.G., GARCIA E.A., ARTONI S. M. B., SGAVIOLI S., SILVA M.D.P., GONÇALVES H. C., PELÍCIA K., 2012 – Remove from marked Records Bone quality of laying hens fed diets supplemented with different vegetable oils. Foregin Title:Qualidade óssea de poedeiras comerciais leves alimentadas com rações suplementadas com diferentes óleos vegetais. ***Journal Veterinária e Zootecnia*** 19, 356-365.
25. GANESAN B., BROTHERSEN C., MCMAHON D.J., 2014 – Fortification of foods with omega-3 polyunsaturated fatty acids. ***Critical Reviews in Food Science and Nutrition*** 54, 98-114.
26. HOFFMAN L.C., MULLER M., S. W. P. CLOETE, AND M. BRAND. 2008 – Physical and sensory meat quality of South African Black ostriches (*Struthio camelus var. domesticus*), Zimbabwean Blue ostriches (*Struthio camelus australis*) and their hybrid. ***Meat Science*** 79: 365-374.
27. HORBAŃCZUK J.O., HUCHZERMAYER F., PARADA R., PŁAZA K., 2004 – Four-legged ostrich (*Struthio camelus*) chick. ***Veterinary Record*** 154, 736.
28. HORBAŃCZUK J.O., KAWKA M., SACHARCZUK M., COOPER R.G., BORUSZEWSKA K., PARADA P., JASZCZAK K., 2007 – A search for sequence similarity between chicken (*Gallus domesticus*) and ostrich (*Struthio camelus*) microsatellite markers. ***Animal Science Papers and Reports*** 25, 283-288.
29. HORBAŃCZUK J.O., SALES J., CELEDA T., KONECKA A., ZIĘBA G., KAWKA P., 1998 – Cholesterol content and fatty acid composition of ostrich meat as influenced by subspecies. ***Meat Science*** 50, 385-388.
30. HORBAŃCZUK J.O., TOMASIK C., COOPER R.G., 2008 – Ostrich farming in Poland – its history and current situation after accession to the European Union. ***Avian Biology Research*** 1, 65-71.
31. HOSSEINI-VASHAN SEYYED JAVAD, ABOLGHASEM GOLIAN, AKBAR YAGHOBFAR., 2014 – Effects of Turmeric Rhizome Powder and Oil Sources on Performance, Immune System and Bone Characteristics in Pre and Post Heat Stressed Broiler Chickens. ***Annual Research & Review in Biology*** 4, 3096-3107.
32. IMMUNDIAGNOSTIK AG., 2006 – Photometric test system for the determination of the total antioxidative status (TAS) in serum and EDTA-plasma. ***Manual*** 11-19.
33. JIANG S., CHENG H. W., CUI L. Y., ZHOU Z. L., HOU J. F., 2013 – Changes of blood parameters associated with bone remodeling following experimentally induced fatty liver disorder in laying hens. ***Poultry Science*** 92, 1443-1453.
34. JANOCHA A., OSEK M., KLOCEK B., SZYMAŃSKA M., 2009 – Effect of adding 25-hydroxycholecalciferol in plant diets with and without fish meal on rearing results and bones of broiler chickens. ***Annals of Animal Science*** 9, 415-423.
35. JOHNSTON N. P., NASH L. L., MACEDA E., DAVIDSON R. T., ARMSTRONG A., 2006 – Effect of feeding diets enriched with either omega-3 or omega -6 polyunsaturated fatty acids on bone characteristics of turkey breeder hens. ***World's Poultry Science Journal*** 62, 342.
36. KAWKA M., HORBAŃCZUK J.O, JASZCZAK K., PIERZCHAŁA M., COOPER R.G., 2012a – A search for genetic markers associated with egg production in the ostrich (*Struthio camelus*)**.** ***Molecular Biology Report*** 39, 7881-7852.
37. KAWKA M., HORBAŃCZUK J.O., SACHARCZUK M., ZIEBA G., ŁUKASZEWICZ M., JASZCZAK K., PARADA R., 2007 - Genetic characteristics of the ostrich population using molecular methods. ***Poultry Science*** 86: 277-281
38. KAWKA M., PARADA R., JASZCZAK K., HORBAŃCZUK J.O., 2012b – The use of microsatellite polymorphism in genetic mapping of the ostrich (*Struthio camelus*) ***Molecular Biology Reports*** 39, 3369-3374.
39. LIU D., VEIT H.P., WILSON J.H., DENBOW D.M., 2003 – Long-term supplementation of various dietary lipids alters bone mineral content, mechanical properties and histological characteristics of Japanese quail. ***Poultry Science*** **82,** 831-839.

40. FISHER P., HOFFMAN L.C., MELLETT F.D., 2000 – Processing and nutritional characteristics of value added ostrich products. ***Meat Science*** 55, 251-254.
41. MAJEWSKA D., JAKUBOWSKA M., LIGOCKI M, TARASEWICZ Z., SZCZERBIŃSKA D., KARAMUCKI T., SALES J., 2009 – Physicochemical characteristics, proximate analysis and mineral composition of ostrich meat as influence by muscle. ***Food Chemistry*** 117, 207-211.
42. MAZZUCO H., MCMURTRY J. P, KUO A. Y., HESTER P. Y., 2005 – The effect of pre- and postmolt diets high in n-3 fatty acids and molt programs on skeletal integrity and insulin-like growth factor-I of White Leghorns. ***Poultry Science*** 84, 1735-1749.
43. MOLLARD R. C., GILLAM M. E., WOOD T. M., TAYLOR C.G., WEILER A., 2005 – (n-3) fatty acids reduce the relase of prostagladin E_2 from bone but do not affect bone mass in obesa (fa/fa) and lean zucker rats. ***Journal of Nutrition*** 135, 499-504.
44. PATWARDHAN D. S., KING A. J., OBERBAUER A. M, HOLLAND T. B., 2011 – Bone measurements of molted layers fed low-salt corn and soybean meal diets containing safflower meal or tomato pomace. ***The Journal of Applied Poultry Research*** 20, 190-196.
45. POŁAWSKA E., HORBAŃCZUK J.O., PIERZCHAŁA M., STRZAŁKOWSKA N., JÓŹWIK A., WÓJCIK A., POMIANOWSKI J., GUTKOWSKA K., WIERZBICKA A., HOFFMAN L.C., 2013 – Effect of dietary linseed and rapeseed supplementation on fatty acid profiles in the ostrich. Part 1. Muscles. ***Animal Science Papers and Reports*** 31, 239-248.
46. POŁAWSKA E., LISIAK D., JÓŹWIK A., PIERZCHAŁA M., STRZAŁKOWSKA N., POMIANOWSKI J., WÓJCIK A., 2012 – The effect of the diet supplementation with linseed and rapeseed on the physico-chemical and sensory characteristics of ostrich meat. ***Animal Science Papers and Reports*** 30, 65-72.
47. POŁAWSKA E., MARCHEWKA J., COOPER R.G., SARTOWSKA K., POMIANOWSKI J., JÓŹWIK A., STRZAŁKOWSKA N., HORBAŃCZUK J. O. 2011 – The ostrich meat – an updated review. II. Nutritive value ***Animal Science Papers and Reports*** 29, 89-97.
48. PUZIO I., JAŚKIEWICZ T., SAGAN A., BIEŃKO M., GRABOŚ D., 2012 – Effects of Cla and Camelina Sativa Seed Oil on Bone Properties in Broiler Chickens. ***Bulletin of the Veterinary Institute in Pulawy*** 57, 287-291.
49. RUBENSON J., D.G. LIOYD T.F. BESIER C.B. HELIAMS, FOURNIER P.A., 2007 – Running in ostriches (*Struthio camelus*): three-dimensional joint axes alignment and joint kinematics. ***Journal of Experimental Biology*** 210, 2548-62.
50. RYBNIK P., HORBAŃCZUK J.O., NARANOWICZ H., ŁUKASZEWICZ E., MALECKI I. A., 2007 – The The teaser female and dummy methods for collecting semen from ostriches (*Struthio camelus*). ***British Poultry Science*** 48, 635-643.
51. SALES J., HORBAŃCZUK J.O., 1998 – Ratite Meat. ***World's Poultry Science Journal*** 54, 59-67.
52. SALES J., HORBAŃCZUK J.O., DINGLE J., COLEMAN R., SENSIK S., 1999 – Carcase characteristics of emus (*Dromaius novaehollandiae*). ***British Poultry Science*** 40, 145-147.
53. TATARA M.R., 2009 – Effect of β-hydroxy-β- methylbutyrate (HMB) administration on volumetric bone mineral density, and morphometric and mechanical properties of tibia in male turkeys. ***Journal of Animal Physiology and Animal Nutrition (Berl)*** 93, 669-77.
54. TATARA M.R, KRUPSKI W., JANKOWSKI M., ZDUŃCZYK Z., JANKOWSKI J., STUDZIŃSKI T., 2011 – Effects of dietary calcium content and vitamin D source on skeletal properties in growing turkeys. ***British Poultry Science*** 52, 718-29.
55. TATARA M.R., KRUPSKI W., MAJCHER P., STUDZIŃSKI T., 2005b – Long-term denervation of the humerus in turkeys as an experimental model for osteopenia. ***Poultry Science*** 84, 718-722.

56. TATARA M.R., PIERZYNOWSKI G. S., MAJCHER P., KRUPSKI W., WAWRZYNIAK-GACEK A., FILIP R., SILMANOWICZ P., STUDZIŃSKI T., 2003 – The influence of alpha-ketoglutarate (AKG) on mineralisation, mechanical and structural properties of ulna in turkey under conditions of osteotomy and denervation. ***Ortopedia Traumatologia Rehabilitacja*** 5, 666-72.
57. TATARA M. R., SIERANT-ROŻMIEJ N., KRUPSKI W., MAJCHER P., ŚLIWA E., KOWALIK S., STUDZIŃSKI T., 2005a – Quantitative computer tomography for the assessment of mineralization of the femur and tibia turkeys. ***Medycyna Weterynaryjna*** 61, 225-228.
58. TATARA M. R., ŚLIWA E., KRUPSKI W., BRODZKI A., PASTERNAK K., 2006 – Ornithine alpha-ketoglutarate increases mineralization and mechanical properties of tibia in turkeys. ***Bone*** 39, 100-105.
59. TATARA M.R., TYGESEN M.P., SAWA-WOJTANOWICZ B., KRUPSKI W., MAJCHER P., HARRISON A.P., 2007 – Bone development: the effect of short term alpha-ketoglutarate administration on long term mechanical properties of ribs in ram lambs. ***Small Ruminant Research*** 67, 179-183.
60. TYKAŁOWSKI B., STENZEL T., KONCICKI A., 2010 – Selected problems related to ossification processes and their disorders in bird. ***Medycyna Weterynaryjna*** 66, 464-469.
61. ZHONG X., GAO S., WANG J.J., DONG L., HUANG J., . ZHANG L.L, Wang T., 2014 – Effects of linseed oil and palm oil on growth performance, tibia fatty acid and biomarkers of bone metabolism in broilers. ***British Poultry Science*** 55, 335-342.