

Chemical composition of bone tissue in broiler chickens intended for slaughter

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ABSTRACT: Selected parameters characterizing the chemical composition of bones were determined in 60 male and 60 female hybrid Ross 308 chickens aged 40 days at the end of the fattening period. The chickens received a complete feed mixture intended for pre-feeding (starter) in a period from the start of the experiment until Day 10, followed by the feed mixture intended for fattening until Day 30 (grower), and the feed mixture for the third phase of fattening, i.e. until Day 40 (finisher). The feed mixtures and drinking water were provided *ad libitum*. At the end of the experiment, chickens were slaughtered and the femur and the tibiotarsus from the right pelvic limb were collected and the following parameters were determined in wet tissue: dry matter (DM), crude protein (CP) ($N \times 6.25$), fat, ash, calcium (Ca), phosphorus (P) and magnesium (Mg); the content of organic matter was calculated. The resultant values were recalculated to 100% of DM. Differences between males and females in the chemical composition of retrieved bones were investigated. The content of CP in wet tissue from both bones ranged from 354.0 ± 11.8 to 365.1 ± 14.3 g/kg being significantly lower ($P < 0.01$) in the tibiotarsus. Differences between sexes were very small. The content of fat in the bones ranged from 16.12 ± 8.71 to 16.17 ± 8.85 g/kg, with higher levels of fat being detected in female chickens (17.16 ± 8.53 to 17.24 ± 8.28 g/kg) as compared to male chickens (15.01 ± 9.05 to 15.19 ± 9.12 g/kg). No statistically significant differences between the femur and the tibiotarsus were detected. The levels of ash ranged from 472.2 ± 13.1 to 480.1 ± 10.83 g/kg, being statistically higher in the tibiotarsus in both male and female chickens ($P < 0.01$; $P < 0.001$). The levels of Ca ranged from 180.4 ± 8.57 to 181.6 ± 12.32 g/kg, showing higher values in both bones from female chickens (181.7 ± 9.17 to 183.8 ± 14.71 g/kg), as compared to male chickens (179.2 ± 7.8 to 179.3 ± 8.91 g/kg). No statistically significant changes were found. The levels of P ranged from 83.17 ± 9.82 to 83.29 ± 7.42 g/kg, with higher levels being observed in female chickens (84.1 ± 9.21 to 89.8 ± 7.47 g/kg), as compared to male chickens (76.5 ± 6.93 to 82.5 ± 5.00 g/kg). The levels of P were significantly higher in the femur ($P < 0.001$) in female chickens and in the tibiotarsus ($P < 0.001$) in male chickens. The Ca:P ratios for both analysed bones was greater in males (femur 2.20, tibiotarsus 2.21), as compared to females (femur 1.98, tibiotarsus 2.09). The mean weight of male and female broiler chickens on Day 40 was $2\ 694 \pm 32.6$ g and $2\ 345 \pm 23.9$ g, respectively. In comparison with the expected performance parameters of ROSS 308 hybrid chickens, the weight increased in both male and female chickens by 8.2% and 10.6%, respectively. Although the chickens grew very quickly, no clinical signs indicating pathological changes in the bone system were found.

Keywords: *Femur; tibiotarsus; ash; calcium; phosphorus; magnesium; body weight*

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The proper function of the skeletal system plays an important role in fast-growing poultry. The genetic selection of broiler chickens to yield greater weight and the development of skeletal muscles do not correspond to the growth of bones. As a result, this may lead to the occurrence of bone deformities

and fractures. This is caused by various factors such as lack of adaptation of the skeleton to increasing body weight (Korver et al., 2004; Whitehead, 2004), inadequate intake of Ca and P, lowered utilization of minerals during fast growth, and genetic factors (Williams et al., 2000). Morphological properties

Table 1. Composition of feed mixtures

Ingredient (%)	Starter (1 to 10 days of age)	Grower (11 to 30 days of age)	Finisher (31 to 40 days of age)
Wheat	24.34	23.55	19.23
Maize	35.00	40.00	40.00
Soybean meal	24.00	18.50	20.00
Fish meal	10.00	10.00	5.00
Wheat feed flour	–	–	5.00
Soybean oil	1.50	3.30	5.43
Monocalcium phosphate	0.37	0.19	0.51
Limestone, powdered	0.90	0.75	1.11
DL-Methionine	0.23	0.17	0.18
NP nove	0.90	0.90	0.80
Biolys 65 ¹	0.16	0.06	0.02
Vitex Q ²	2.50	2.50	2.50
Feed salt	0.10	0.08	0.22
Analysed composition (g/kg)			
Dry matter	883.60	885.60	887.10
Crude protein (N × 6.25)	242.80	220.10	199.80
Fat	45.50	64.60	82.70
Fibre	26.10	24.90	26.10
Ash	56.60	50.60	51.90
Ca	10.58	10.45	10.00
P	6.22	6.31	6.35
Mg	2.42	1.46	1.94
ME _N (MJ/kg), calculated	12.6	13.29	13.5

the premix of specifically active substances used by the producer contained: vitamin A 1 600 000 IU; vitamin D₃ 500 000 IU; alpha-tocopherol 10 000 mg; vitamin K₃ 300 mg; vitamin B₁ 800 mg; vitamin B₂ 1 300 mg; vitamin B₆ 600 mg; vitamin B₁₂ 3 mg; biotin 30 mg; folic acid 500 mg; niacinamide 6 000 mg; calcium pantothenate 2 500 mg; betaine 50 000 mg; butylhydroxytoluene 3 400 mg; propyl gallate 1 200 mg; ethoxyquin 540 mg; ferrous sulphate monohydrate 10 000 mg; manganese oxide 16 000 mg; zinc oxide 16 000 mg; copper sulphate 1 700 mg; potassium iodide 200 mg; sodium selenite 30 mg; cobalt sulphate 50 mg; phytase 50 000 FTU; glucanase 24 000 BGU; xylanase 1 100 000 EXU

¹Biolys 65[®] – L-lysine sulphate, a fermentation product with a minimum lysine content of 50.7% (Evonik Degussa GmbH)

²feed yeast (Biocel Paskov a.s., Czech Republic)

and the internal structure of bones should ensure optimum counterbalance against forces associated with the supportive and locomotor function of the skeleton not only in birds but also in the other animal species (Ferretti et al., 1993a,b; Kanis, 1994; Webster, 2004; Tatara et al., 2006).

About 70 to 80% of bone mass is determined genetically while the resulting 20 to 30% is attributed to external factors of which a diet is the most important factor (Eastell and Lambert, 2002) as it has a significant effect on bone mineralization (Huyghebaert, 1997). Apart from the metabolic side-products of digestion and compounds synthesised in the intestine, vitamins, minerals or any other nutrient deficiency or excess are also among the important nutritional factors that may cause changes in the pelvic limbs. Vitamin D, Ca, and P are nutrients that play a major role in this respect. Increased levels of both Ca and available P resulted in increased levels of minerals in the tibiotarsus (Huyghebaert, 1996; Onyango et al., 2003). Vitamins A, E, C, folic acid and some B vitamins are also important (Oviedo-Rondon et al., 2006; Waldenstedt, 2006). Huyghebaert (1997) reported that the Ca:P ratio of 2.8 is optimal to reach the maximum level of ash in the tibiotarsus, which corresponds to a demand for Ca and P of 1.04% and 0.36%, respectively (i.e. 10.4 g of Ca and 3.6 g of P in the feed mixture).

The proper structure and function of skeletal system are among the preconditions for successful fattening of broiler chickens and information on the skeleton chemical composition can contribute to that. The aim of the study was to determine selected parameters of bone metabolism for the long bones of pelvic limbs (*femur* and *tibiotarsus*) in 40-day-old male and female broiler chickens reared under defined experimental conditions and an optimal diet.

MATERIAL AND METHODS

Birds, diets and experimental design

The experiment was performed with 120 Ross 308 sexed broiler chickens (60 males and 60 females). One-day-old chickens were allotted to two groups according to sex. Each group consisted of 3 replicate pens with 20 birds per pen. Stocking density was 15 birds per 1m² of floor space. Hanging tube feeders and automatic round drinkers provided feed and water *ad libitum*. During the experiment, a photoperiod of 23L:1D was maintained and the temperature

was decreased from 27 to 24°C by 0.3°C per day. The chickens were reared in the accredited experimental poultry house of the University of Veterinary and Pharmaceutical Sciences in Brno, Czech Republic.

During the experiment, chickens received the following complete feed mixtures: starter from 1 to 10 days of age, grower from 11 to 30 days of age and finisher from 31 to 40 days of age. Feed mixtures and drinking water were provided *ad libitum*. Ingredient and nutrient compositions of the feed mixtures are presented in Table 1.

During the experiment, chicken health was monitored. At the end of the fattening period, chickens aged 40 days were weighed individually and slaughtered. The femur and the tibiotarsus were collected from the right pelvic limb.

Chemical composition of bones

The bones were freed of soft tissue, partially defatted with ether for at least 24 hours, ashed at 550°C and analyzed. The moisture content (dry matter) was determined gravimetrically as the residue remaining after drying the samples, crude protein was assessed by measuring nitrogen content (N × 6.25) using a Buchi analyzer (made by Centec automatika, Ltd.). Fat content was measured by an ANKOM^{XT10} Fat Analyzer (made by O.K. Servis BioPro), ash was determined by the gravimetric method after heating to 550°C. Ca, P and Mg were assessed by ashing, extraction and titration, all of these analyses were performed under conditions outlined in AOAC (2001). The values found were related to 100% DM. The content of organic matter (OM) was calculated.

Statistical analysis

Data were analysed using ANOVA followed by multiple comparison using the Tukey-HSD test (Zar, 1999) to detect statistically significant differences between groups. The results were statistically processed using the Unistat 5.1 programme (Unistat Ltd., UK).

RESULTS AND DISCUSSION

The levels of DM, CP, fat, ash, Ca, P, Mg and OM in wet bone tissue and 100% DM (regardless of the

Table 2. The levels (g/kg) of selected parameters of bone metabolism of the femur and tibiotarsus in 40-day-old broiler chickens (wet bone tissues and 100% DM)¹

Parameter	Wet tissue		100% DM	
	femur	tibiotarsus	femur	tibiotarsus
Dry matter	901.1 ± 2.96	899.3 ± 5.75**	1 000.0	1 000.0
Crude protein	365.1 ± 14.30	354.0 ± 11.80***	405.3 ± 18.10	393.5 ± 13.00***
Fat	16.1 ± 8.71	16.2 ± 8.85	17.9 ± 9.64	18.0 ± 9.84
Ash	472.2 ± 13.10	480.1 ± 10.83***	525.1 ± 14.27	533.7 ± 12.93***
Ca	180.4 ± 8.57	181.6 ± 12.32	200.1 ± 9.38	202.4 ± 12.03
P	83.2 ± 9.82	83.3 ± 7.42	92.2 ± 10.98	92.7 ± 8.55
Mg	2.2 ± 0.20	2.1 ± 0.20**	2.4 ± 0.24	2.4 ± 0.23*
Organic matter	429.0 ± 13.46	419.2 ± 12.00***	474.9 ± 14.27	466.3 ± 12.93***

¹data are means ± SD from 120 chickens (60 males and 60 females)

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

sex) are listed in Table 2. The levels of CP in studied bones (in wet tissue) ranged from 354.0 ± 11.80 to 365.1 ± 14.30 g/kg being statistically lower in the tibiotarsus ($P < 0.01$). For example Da Silva et al. (2001a) reported for 14-day-old male chickens that the average level of CP in the femur and the tibiotarsus was 300.35 g/kg. The content of fat in bones ranged from 16.1 ± 8.71 to 16.2 ± 8.85 g/kg, being higher in female chickens (17.16 ± 8.53 to 17.2 ± 8.28 g/kg) (Table 3) as compared to male chickens

(15.0 ± 9.05 to 15.2 ± 9.12 g/kg) (Table 4). No statistically significant differences between the femur and the tibiotarsus were found. The content of ash in the tibiotarsus original mass (480.1 ± 10.83 g/kg) was statistically higher, as compared to the femur (472.2 ± 13.1 g/kg) in both males ($P < 0.001$) and females ($P < 0.01$). This finding corresponds well with the results reported by Da Silva et al. (2001b), who also found in males during fattening that the levels of ash in the femur (406.0 g/kg) were lower

Table 3. The levels (g/kg) of selected parameters of bone metabolism of the femur and tibiotarsus in 40-day-old female broiler chickens (wet bone tissues and 100% DM)¹

Parameter	Wet tissue		100% DM	
	femur	tibiotarsus	femur	tibiotarsus
Dry matter	901.3 ± 2.48	895.6 ± 2.92***	1 000.0	1 000.0
Crude protein	365.5 ± 13.15	351.4 ± 11.15***	404.1 ± 16.29	392.1 ± 13.21***
Fat	17.2 ± 8.28	17.2 ± 8.53	19.1 ± 9.17	19.1 ± 9.50
Ash	471.9 ± 12.45	478.3 ± 10.30**	523.5 ± 14.13	533.7 ± 13.29***
Ca	181.7 ± 9.17	183.8 ± 14.71	201.4 ± 10.08	206.3 ± 12.64*
P	89.8 ± 7.47	84.1 ± 9.21***	99.6 ± 8.21	94.0 ± 10.67**
Mg	2.21 ± 0.20	2.17 ± 0.19	2.4 ± 0.24	2.4 ± 0.22
Organic matter	429.4 ± 13.36	417.2 ± 11.47***	476.5 ± 14.13	466.3 ± 13.29***

¹data are means ± SD from 60 female broiler chickens

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Table 4. The levels (g/kg) of selected parameters of bone metabolism of the femur and tibiotarsus in 40-day-old male broiler chickens (wet bone tissues and 100% DM)¹

Parameter	Wet tissue		100% DM	
	femur	tibiotarsus	femur	tibiotarsus
Dry matter	900.9 ± 3.39	903.0 ± 5.49*	1 000.0	1 000.0
Crude protein	364.8 ± 15.50	356.7 ± 11.97**	406.6 ± 19.84	395.0 ± 12.64***
Fat	15.0 ± 9.05	15.2 ± 9.12	16.6 ± 10.08	16.8 ± 10.10
Ash	472.4 ± 13.82	481.8 ± 11.14***	525.8 ± 14.43	533.7 ± 12.68**
Ca	179.2 ± 7.80	179.3 ± 8.91	199.0 ± 8.51	198.6 ± 10.10
P	76.5 ± 6.93	82.5 ± 5.00***	84.9 ± 8.18	91.4 ± 5.48***
Mg	2.2 ± 0.21	2.08 ± 0.20**	2.4 ± 0.25	2.3 ± 0.21**
Organic matter	428.5 ± 13.70	421.2 ± 12.30**	474.3 ± 14.43	466.3 ± 12.68**

¹data are means ± SD from 60 male broiler chickens

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

than those in the tibiotarsus (407.3 g/kg). The levels of Ca in both bones were in the range from 180.4 ± 8.57 to 181.6 ± 12.32 g/kg, being higher in females (181.7 ± 9.17 to 183.8 ± 14.71 g/kg), as compared to males (179.2 ± 7.80 to 179.3 ± 8.91 g/kg). No statistically significant changes were found. The levels of P in both bones ranged from 83.2 ± 9.82 to 83.3 ± 7.42 g/kg, showing higher values in females (84.1 ± 9.21 to 89.8 ± 7.47 g/kg), as compared to males (76.5 ± 6.93 to 82.5 ± 5.00 g/kg). The mean levels of P in the femur were statistically higher ($P < 0.001$) in female chickens, while in male chickens they were statistically higher in the tibiotarsus ($P < 0.001$). The ratio of Ca:P for both studied bones was greater in males (femur 2.20, tibiotarsus 2.21), as compared to females (femur 1.98, tibiotarsus 2.09). A lower Ca:P ratio (1.80–1.95) was reported by Da Silva et al. (2001a) in males during growth, whereas Skinner and Waldroup (1995) found Ca:P ratios of 1.98 and 1.94 in 42-day-old male chickens and female chickens, respectively. The levels of DM, CP, Mg, and OM in the wet bone tissue of the tibiotarsus were statistically lower as compared to the femur ($P < 0.01$; $P < 0.001$), whereas the levels of ash ($P < 0.001$) were statistically significantly higher.

The ash content in the tibiotarsus recalculated to 100% DM (Table 3) was significantly higher compared to the femur (53.4% vs. 52.4%). The content of Ca in ash from the tibiotarsus and the femur reached 38.2% and 37.8%, respectively. The P levels

in ash were 17.4% in tibiotarsus and 17.6% in femur. The content of CP in the tibiotarsus was significantly lower ($P < 0.001$) compared to the femur.

Female chickens (Table 3) showed significantly lower ($P < 0.01$; $P < 0.001$) levels of DM, CP, P, and OM in wet bone tissue of the tibiotarsus compared to the femur, whereas the levels of ash in the tibiotarsus were significantly higher ($P < 0.01$). The content of ash in DM (Table 3) in the tibiotarsus was significantly higher ($P < 0.001$) compared to the femur (53.4% vs. 52.4%). The content of Ca in ash in the tibiotarsus and the femur was 38.6% and 38.5%, respectively ($P < 0.05$). The levels of P in the tibiotarsus and the femur ash were 17.6% and 19.0%, respectively. Da Silva et al. (2001b) reported 37.01% of Ca and 20.55% of P in ash from the tibiotarsus. Venäläinen et al. (2006) found that the levels of ash, Ca and P in the tibiotarsus from males were higher than those in females. By contrast, Hulan et al. (1985) detected lower levels in male chickens, which are similar to our findings. No statistically significant differences were found for fat and Mg.

In males (Table 4), the levels of CP, Mg, and OM in wet bone tissue were significantly lower ($P < 0.01$; $P < 0.001$) in the tibiotarsus compared to the femur while the levels of DM, ash, and P were higher ($P < 0.05$; $P < 0.001$; $P < 0.001$, respectively). Related to 100% DM (Table 4), the level of ash in the tibiotarsus was also higher (53.4%), as compared to the femur (52.6%). The levels of Ca in ash from the

tibiotarsus and the femur were 37.2% and 37.8%, respectively, while P levels were 17.1% and 16.1%, respectively ($P < 0.001$). No statistically significant differences were found for fat and Ca.

The mean body weight of 40-day-old broiler chickens at the end of the fattening period was $2\ 694 \pm 32.6$ g (males) and $2\ 345 \pm 23.9$ g (females). According to the technological procedure for fattening Ross 308 combination broilers (Anonymous, 2002), 40-day-old males and females should weigh 2 489 g and 2 120 g, respectively. The weights of male and female chickens in our study were 8.2% and 10.6% higher compared to the expected weights. Although the experimental chickens grew more quickly than expected, no clinical signs indicating pathological changes in the skeletal system were detected.

CONCLUSIONS

The bone metabolism parameters of long bones of pelvic limbs (femur and tibiotarsus) were determined in wet tissue and in 100% DM in 40-day-old Ross 308 broiler chickens reared under defined experimental conditions and an optimal diet. Crude protein content was significantly higher in the femur compared to the tibiotarsus. The bones had a similar fat content but the tibiotarsus had significantly higher ash content than the femur. The content of Ca was higher in the tibiotarsus. The bones did not differ in Mg content and also the Ca:P ratio was similar in both bones (2.17 vs. 2.18). The mean body weight of Ross 308 broiler chickens aged 40 days was higher than the performance objectives given by the stock producer. Although the chickens grew very quickly, no clinical signs indicating pathological changes in the bone system were found.

The nutritive composition of diets produced the fast growth of chickens. Clinical signs indicating pathological changes in the bone system were not found during the fattening.

The female chickens had higher contents of Ca, P and Mg in both the femur and the tibiotarsus compared to the male chickens, with ash content being lower in the females.

The results showed that, regardless of the sex, the tibiotarsus may be more suitable to examine the effect of a diet on bone mineralization as it shows significantly higher levels of both ash, and Ca and P.

The established differences can stimulate consideration in determination of Ca and P requirements with regard to the sex.

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