# Metrical and Mechanical Parameters and Chemical Composition and Histological Structure of Bones and Mineral Substances Retention in Chickens Fed Diet Containing Different Amounts of Calcium and Phoshorus

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## Introduction

The bone quality and motorical ability of fast growing broilers are the very important aspects of meat-type poultry breeding. Recommendations for optimum level of calcium and phosphorus in broiler diets depends on many factors, such as genotype and age of birds, kind of cereal grains applied in diets (NSP and phytate content), energy concentration, sources of dietary phosphorus and calcium, supplementation with enzymes, especially phytase addition. According to EC recommendations (2003), the starter diets for broiler chickens should contain 0.65 to 0.78 % of total phosphorus; the phosphorus quantity in grower and finisher mixtures should vary at limits of 0..65 % and within 0.57 to 0.67 %, respectively. The NRC (1994) and Polish recommendations (2005) for phosphorus content in broiler starter diets gives the values similar to the EU data - for total P 0.64-0.68 and for P-available 0.38 to 0.45 %. The recommended amounts of calcium vary between 0.85 and 0.97 %. Data presented for different hybrids gives diversified values related to the requirement for Ca and P e.g. Cobb-Vantress Hybrid provides the amount 0.84-0.90 % of calcium and 0.40-0.45 % of available phosphorus during all growth phases, the Hubbard ISA (2002) provides the amounts of 1.1 % of Ca and 0.45 % of P available and Ca:P available ratio as 2.44:1.

The purpose of presented investigation was to evaluate the mechanical, metrical and chemical parameters as well as histological structure of tibia of chickens fed diets differing in the Ca:P ratios. Moreover, the effect of this parameter on accretion of mineral elements in chickens during early growth phase, when the important processes that decide about the correct formation of bone structure (Whitehead, 2005) and their strength and elasticity occur have also been evaluated.

## Material and methods

One hundred and thirty two one-day old Hubbard Flex male broiler chickens with average initial body weight ca. 39.5 g (±0.91) were randomly divided to four groups and were kept in battery cages under optimal environmental conditions. Birds fed feed mixtures in mash form (starter from 1st to 14th days of life and grower from 15th to 28th day) containing 215/205 g of crude protein, 12.3/11.6 g lysine and 9.3/9.0 g total sulphur amino acids per kg of mixture, respectively. The apparent metabolizable energy (AME) value amounted ca. 12.0 MJ per kg of mixture. Isoenergetical and isoprotein feed mixtures offered ad libitum were diversified in calcium and phosphorus level (Table 1).

The dynamic of bone mineralisation proccesses was estimated in chickens. On day 1, 7, 14, 21 and 28 post hatch, chickens were weighed individually then eight birds from each day and treatment (2-3 birds per replication) were randomly selected (with average weight within replication), killed by cervical dislocation then the both legs were prepared, the muscles were removed then the tibia bones were cleaned and stored for estimation of quality parameters (16 bones per each treament). Four fresh bones from each group and age of chickens were separated for histology assayes, next 6 bones were separated for mechanical, metrical measurements and 6 bones for chemical determinations. In fresh bones the mechanical parameters as: strength in maximal force and deflection were determined using the INSTRON 5544 (USA) apparatus. The technique of measurements was in details presented by Jamroz et al.(2004). In defatted (ether extract during 24 hours, according to Soxhlet method, in 45oC)

dried bones the metrical parameters such as length and weight were estimated, and crude ash and the content of Ca, P and Mg were determined.

The histological examinations were carried out in the samples taken from the epiphysial zone, metaphysial area as well as from the diaphysis of tibia. Prepared fragments of the bone were fixed in the 4 % aqueous solution of formalin buffered with calcium carbonate, for 72 hours. After this procedure segments were rinsed in the tap water and decalcified in the 10 % solution of EDTA during 7 days and then with the mixture of formic acid and sodium citrate during 14 days. Prepared biological material was dehydrated in the alcoholic series and paraffin embedded. Material was cutted into scraps of 7 μm thick and stained with hematoxylin and eosin according to the Delafield method. The microphotographies were obtained using the Canon PS66 camera.

In days 8-11 (starter period; 3 cages, each with 8 birds per treatment) and 25-28 (grower period; 3 cages, each with 2-3 birds per treatment) of chickens life the balances of calcium, phosphorus, magnesium were performed. The feed intake was controlled and excrements were quantitatively collected during the period of four days, in them the feed intake and both quantity and chemical composition of feed and excrements were recorded.

The chemical composition of both diets and tibia bones were determined according to standard methods AOAC (2005). All obtained data were evaluated statistically by one or two-factorial ANOVA (mineral composition of epi- and diaphysis) using StatSoft Statistica® software (2005). All procedures that were carried out with animals were approved by the Local Ethical Commission.

**Results**

During grower-experimental time in treatments III and II fed mixtures with low phosphorus level (3.7 g/kg) the body weight of chickens was significantly ($P<0.05$) lower in comparison to treatments I and IV.

In mechanical parameters of tibia bones were registered significantly ($P<0.05$) lower values for strength in maximal force on 7 (in group IV) and 28 day of life in treatment IV and II by 9 g Ca; 3.7 or 4.5 g P/kg. The best results were noted in 7 day of life in chickens from treatment II, and in age of 28 days in treatment I. In total the higher values of maximal strenght were estimated in 28 day of life in treatments fed with higher calcium concentration in diets I and III ($P<0.01$) (Table 2). No clear tendency was registered in parameters of elasticity of bones and in maximal deflection in comparison to maximal strength. In measurements conducted in day 7 post hatch, only in chickens from treatment IV the higher elasticity value and lower strenght were observed. On 28 day of life the lower values of elasticity of bones were stated in chickens from group IV and II (with lower Ca level); paralelly lower bones strength were registered. No other mechanical and physical parameters such as vectors of elasticity, maximal percent of bone deflection and breaking work were significantly affected by Ca and P level in diets. The use of the diets containing higher calcium (11.0 g/kg) and phosphorus (4.5 g/kg) level (treatments I and IV) positively influenced the mechanical parameters (strength and elasticity) of tibia bone of 28 day old chickens in comparison to other treatments.

The development of bones, evaluated considering the metrical parameters of tibia, was insignificantly different in chickens from all treatments on 7, 14, 21 day post hatch. On day 28 of life significantly ($P<0.05$) higher tibia lengths and their weight were registered in chickens fed diets with higher calcium level (11.0 g/kg) as compared to treatments II and IV.

On day 28 post hatch a higher concentration of crude ash has accompanied the higher calcium level in ash content in tibia. Using in chickens feeding of diets with the lower calcium but higher phosphorus level (4.5 g/kg) (IV) had not increased the phosphorus content in crude

ash of tibia. From the other side in chickens fed diets contained the lower phosphorus level (3.7 g/kg) (III) with high Ca level decreased phosphorus concentration in tibia ash of 28 day old chickens was observed. The calcium/phosphorus ratio in tibia ash during experimental time was from in mean 1.34 on one week old chickens to 1.51 in 28 days old birds.

The best Ca - accretion was noted in treatment IV (9 g Ca, 4.5 g P-available; $P<0.05$). Generally in both balance periods the feeding of chickens mixtures with lower phosphorus level 3.7 g/kg (treatment II and III) increased the phosphorus accretion in comparison to treatments fed diets with higher phosphorus content, (4.5 g/kg). No significant diversified and with great treatment variance in accretion of Mg was stated. In very young birds the deposition of Mg in chicks varied between 20-27 %, in older birds was 15.5-20.5 % of Mg-intake.

As an effect of conducted histological examinations it was stated that the most essential changes, in comparison to the control birds, were observed in chickens from treatments II and III. Analysis of the area between the epiphysis and diaphysis of the tibial bone allow to conclude that on the 7th day post hatch in chicks from the treatment II the fully-formed lamellas of bone of the osseous spongy tissue were observed. Among the trabeculas created from the lamellas of bone the reds (haemopoietic) marrow occur. Such formed osseous trabecula give the picture of the modifying and rebuilding structures in comparison to the control group.

In tibia of chickens from treatment III, the similar structure of the bone tissue as in treatment II was observed. The attention however should be paid to the not such fully formed trabecula. Moreover the enlarged areas of the osteogenesis on the endosteal side were observed. The surface of trabecula shows the differentiating osteoblasts, what indicate the both bone growth and development.

In the histological pictures observed in bones from treatment IV not such profitable from point of view of postnatal osteogenesis structures could be seen. Trabecula are thicker and the osteogenesis is expressed poorly.

On the day 14 post hatch in bones of birds from treatment II the higher degree of formation and stabilization of structures formed the osseous trabecula is stated. The bone marrow present in the spaces created from the trabecula, indicate the essential developmental progression. This indicates the intensive processes of the haemocytopoiesis.

In birds from treatment III, on the 14 day old experiment the most complete stabilization of structures involved of bone tissue could be noted. The bone marrow present among the trabecula of osseous spongy tissue shows the intensive haemocytopoiesis. The histological structure of the bone tissue observed in chickens from treatment IV on 14 day of experiment shows the similar picture as observed in the same treatment on day 7 of chickens life.

In treatment II, in 28 day old chickens the further developmental progression and osteogenesis stabilization was observed. The bone tissue of the examined areas represents structures that are characteristic for the fully developed bone. In birds from treatment III the similar pictures as in II were observed and in treatment IV the lasting and advanced process of osteogenesis with the areas of the bone modeling and reconstruction could be seen.

As a final conclusion it could be stated that the best bone quality and its chemical composition was obtained in chickens fed diet with 11 g Ca and 4.5 g P-available/kg mixture, however ability of adaptation to the lower levels of Ca and P was observed. Some negative effects on bone quality observed in first 3 weeks post hatch, were not stated on day 28.

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Table 1. Composition of the experimental feed mixtures

| | Treatment groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | I | | II | | III | | IV | |
| | Starter | Grower | Starter | Grower | Starter | Grower | Starter | Grower |
| | Components (g/kg) | | | | | | | |
| Maize | 329.6 | 355.9 | 337.8 | 363.1 | 332.3 | 363.1 | 336.8 | 367.3 |
| Wheat | 286.0 | 295.3 | 286.0 | 295.3 | 286.0 | 290.0 | 286.0 | 290.0 |
| Soya bean meal | 310.0 | 280.0 | 310.0 | 280.0 | 310.0 | 280.0 | 310.0 | 280.0 |
| Soya oil | 30.0 | 24.0 | 28.0 | 23.0 | 28.0 | 23.0 | 28.0 | 23.0 |
| L-Lysine | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 |
| DL-Methionine | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Limestone | 17.2 | 17.3 | 14.0 | 14.1 | 19.5 | 19.5 | 12.0 | 12.0 |
| Ca-phosphate | 14.5 | 14.8 | 11.5 | 11.8 | 11.5 | 11.7 | 14.5 | 1.50 |
| Salt | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Premix[1] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| | Estimated nutrients (g/kg) | | | | | | | |
| Crude protein | 215.6 | 204.9 | 216.5 | 205.1 | 215.8 | 205.1 | 214.9 | 205.6 |
| Lysine | 12.31 | 11.61 | 12.31 | 11.61 | 12.31 | 11.61 | 12.31 | 11.61 |
| TSAA[2] | 9.21 | 9.02 | 9.21 | 9.02 | 9.21 | 9.02 | 9.21 | 9.02 |
| Treonine | 7.60 | 7.20 | 7.60 | 7.20 | 7.60 | 7.20 | 7.60 | 7.20 |
| Tryptophan | 2.42 | 2.20 | 2.42 | 2.20 | 2.42 | 2.20 | 2.42 | 2.20 |
| $AME_N$ (MJ/kg)[3] | 12.04 | 12.03 | 12.06 | 12.05 | 12.04 | 12.04 | 12.07 | 12.04 |
| Ca | 11.01 | 11.02 | 9.04 | 9.01 | 11.03 | 11.03 | 9.02 | 9.04 |
| P total | 6.97 | 6.97 | 6.30 | 6.31 | 6.29 | 6.27 | 6.99 | 7.04 |
| P avail. (calcul.) | 4.49 | 4.51 | 3.71 | 3.70 | 3.70 | 3.71 | 4.50 | 4.56 |
| Mg | 2.00 | 1.95 | 1.98 | 1.92 | 2.03 | 1.98 | 1.96 | 1.90 |
| Na calculated | 1.75 | 1.75 | 1.75 | 1.75 | 1.75 | 1.75 | 1.75 | 1.75 |
| Cl | 2.21 | 2.19 | 2.21 | 2.20 | 2.21 | 2.19 | 2.21 | 2.19 |

[1] added per kilogram of diet: retinyl palmitate, 5.5 mg; cholecalciferol, 0.05 mg; DL-α-tocopheryl acetate, 20 mg; menadione, 3 mg; thiamin, 2.5 mg; riboflavin, 4.5 mg; pyridoxine, 4 mg; cyanocobalamin, 0.015 mg; nicotinic acid, 25 mg; Ca-pantothenate, 8 mg; folic acid, 1.2 mg; choline chloride, 450 mg; DL-methionine, 1.0 mg; Mn, 74 mg as MnO; Fe, 30 mg as $Fe_2SO_4 \cdot H_2O$; Zn, 45 mg as ZnO; Cu, 4 mg as CuO; Co, 0.4 mg as $CoSO_4$; iodine, 0.3 mg as KI;

[2] TSAA - Total Sulphur Amino Acids

[3] calculated according to European Table of Energy Values for Poultry Feedstuffs (1989), 3rd Edition, WPSA and the chemical composition of the diets according to GfE Empfehlungen DLG (1999).

Table 2. Chemical composition, metrical and mechanical parameters of bones and retention of Ca, P and Mg in chickens

| Parameters | Ca/P-available level in feed mixtures (g/kg) | | | |
|---|---|---|---|---|
| | I<br>11/4.5 | II<br>9/3.7 | III<br>11/3.7 | IV<br>9/4.5 |
| Chemical composition of tibia bone on day 28 post hatch | | | | |
| Crude ash (CA) (%) | 40.5a | 38.8ab | 37.9b | 37.8b |
| Ca (g/kg CA) | 265.0a | 259.3b | 251.0b | 257.7b |
| P (g/kg CA) | 171.8a | 171.9a | 166.2b | 175.2a |
| Metrical parameters of bone on day 28 post hatch | | | | |
| Length (cm) | 7.93a | 7.59a | 7.75ab | 7.56b |
| Weight (g) | 4.49a | 3.77b | 4.11ab | 3.93b |
| L/W index | 1.77a | 2.02b | 1.88a | 1.92ab |
| Mechanical parameters of bones | | | | |
| Strength in maximal force (N) | | | | |
| day 7 post hatch | 26.05a | 27.69a | 25.76ab | 23.07b |
| day 28 post hatch | 289.9A | 181.2B | 206.2AB | 172.0B |
| Elasticity – maximal bone deflection (N/m) | | | | |
| day 7 post hatch | 45392a | 46122a | 46255a | 49630b |
| day 28 post hatch | 298448a | 275413a | 283225a | 263795 |
| Accretion of minerals (in % of Ca, P, Mg intake) | | | | |
| Day 11 post hatch | | | | |
| Ca | 73.5ab | 71.4a | 73.5ab | 76.5b |
| P | 56.3ab | 62.5b | 59.4ab | 58.4ab |
| Mg | 20.4 | 23.0 | 23.9 | 27.0 |
| Day 28 post hatch | | | | |
| Ca | 69.3a | 68.3a | 66.7b | 71.3a |
| P | 47.8ab | 51.2b | 52.6b | 43.5a |
| Mg | 15.5 | 17.1 | 20.5 | 18.7 |

The average values in the same rows marked with a,b differ significantly by P<0.05.
The average values in the same rows marked with A,B differ significantly by P<0.01

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