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COMPARISON OF THE EFFECT OF A STANDARD INCLUSION LEVEL OF INORGANIC ZINC TO ORGANIC FORM AT LOWERED LEVEL ON BONE DEVELOPMENT IN GROWING MALE ROSS BROILER CHICKENS*

Ewa Tomaszewska[1♦], Piotr Dobrowolski[2], Małgorzata Kwiecień[3], Agata Wawrzyniak[4], Natalia Burmańczuk[1]

[1]Department of Animal Physiology, University of Life Sciences, Akademicka 12, 20-950 Lublin, Poland
[2]Department of Comparative Anatomy and Anthropology
Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland
[3]Department of Animal Nutrition, Institute of Animal Nutrition and Bromatology, University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland
[4]Department of Animal Anatomy and Histology, University of Life Sciences, Akademicka 12, 20-950 Lublin, Poland
[♦]Corresponding author: ewaRST@interia.pl

Abstract

The purpose of this study was to compare the effect of a standard inclusion level of inorganic zinc to organic form at lowered level on the bone development in growing male Ross 308 chickens, assessed on the basis of mechanical, geometric, and histomorphometric parameters of limb bone, and bone zinc content, as well as hormones of somatotropic axis. A total of 80 one-day-old male Ross broiler chickens were randomly allocated to 2 groups of 40 chickens each. The control group was fed with a corn-soybean meal basal diet providing the recommended zinc amount (100 mg×kg^{-1}) from zinc oxide, and the experimental group was supplemented with glycinate chelate providing 25% of the total requirement of the microelement recommended for Ross 308 broiler chicks. The mechanical and histomorphometric parameters and geometry of tibia were determined as well as the serum concentration of growth hormone, IGF-1, osteocalcin and leptin. The serum concentration of Zn, Cu, Ca, Mg, Fe, P and zinc bone content were determined. The results showed that birds fed with the diet supplemented with organic zinc in the amount of 25% of the recommended amount did not exhibit weight and general growth disorders and had an unchanged concentration of growth hormone, leptin, and IGF-1. The serum concentration of Zn, Cu, Ca, Mg, Fe, P did not differ between groups. The contents of zinc detected in bones in the controls and the group supplemented with the organic source did not differ as well. Although tibial mechanics and geometry remained unchanged, histomorphometry revealed a disproportionately large osteoporotic bone. The changes in tibial trabecular bone as a result of the diet supplemented with glycinate chelate only in 25% of the total requirement of the microelement recommended for Ross 308 broiler chicks seems to be insufficient for tibia development.

Key words: chicken, zinc chelate, tibia, histomorphometry, mechanics

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Zinc (Zn) is an essential micronutrient with particular physiological functions in all living systems. It should be commonly supplemented in poultry diet, because many natural feed ingredients are Zn-deficient. Zinc is necessary for normal growth and bone development, maintenance of immunological functions, feathering, enzyme structure and function, and appetite regulation for all avian species (Cao et al., 2002; Park et al., 2002). Thus, the feed industry has recently made increasing use of organic zinc mineral supplements, which are commonly added to all formulated poultry diets in 0.012–0.018% on a total-weight basis (Cao et al., 2002; Park et al., 2002).

There is an increasing demand for poultry worldwide, especially for the meat of broiler chickens, production of which is more profitable (El-Husseiny et al., 2012; Sahraei et al., 2012). Broiler chickens live for a very short time and their skeletal system undergoes intensive growth and is exposed to a constant influence of many factors. Among the several causal factors, there are unstable and unbalanced nutrition energy, protein and minerals, poisoning and hormonal disorders, and genetic predisposition (Cook, 2000). Moreover, because of the sex-specific heavier body weights, the overloading or abnormal loading conditions of pelvic limb bones, structural damage, and deformities are regularly observed in males (Śliwa et al., 1996; Tatara et al., 2005). The proper function of the skeletal system is needed for poultry production. Bone deformities, lesions, mineralization, and fractures are a result of insufficient adaptation of the skeleton to high body weight and can be observed in leg bones growing faster than other bones in the body. All these finally result in economic losses.

Traditional inorganic mineral salts include sulphates, oxides, and carbonates. Inorganic forms of minerals are used at ca. 10-fold higher doses than the recommended ones in order to avoid trace mineral deficiency and to allow chickens to reach their optimal genetic growth (El-Husseiny et al., 2012). Therefore, at present, farm animals are commonly fed with highly concentrated diets that provide an excess of minerals to maximize performance. On the other hand, this practice is harmful to the environment. Moreover, the availability of trace minerals from these sources differs. Sulphates and oxides have the highest bioavailability (El-Husseiny et al., 2012). Some studies report greater bioefficacy of organic sources (Świątkiewicz et al., 2001; Andersen, 2004; Sahraei et al., 2012). Amino acid chelates are reported to have significantly higher absorption rates from the intestine due to their unique chemical structure compared to inorganic salts (Marchetti et al., 2000; Cao et al., 2002; Tomaszewska et al., 2014). Thus recently, there has been increased interest in new generation chelates, i.e. glycinate chelates. Studies by Kwiecień (2012 and 2014) show that the use of Cu in the form of glycinate compounds at a much reduced level instead of Cu added in the form of sulfate according to recommended dose did not cause a deterioration in the physical, chemical, mechanical and morphometric properties of femur in chickens.

However, the number of scientific analyses involving the use of glycinate chelates in feeding of broiler chickens and their effect on the bone structure forming processes is limited (Kwiecień, 2012; Kwiecień et al., 2014). With regard to the important role of Zn in the development of bones, it is hypothesized that the use of

Zn in a more assimilable form might improve the development of the skeletal system in the early stages of life. Moreover, a high bioavailability of Zn from chelates causes that an excretion of microelement to the environment is limited and there is less interest in zinc oxide. For this reason, zinc oxide is displaced from practice and our study lacks the group supplemented with inorganic zinc at a much reduced level.

The purpose of this study was to compare the effect of a standard inclusion level of inorganic zinc to organic form at lowered level on the bone development in growing male Ross 308 chickens, assessed on the basis of mechanical, geometric, and histomorphometric parameters of limb bone, and bone zinc content, as well as hormones of somatotropic axis.

Material and methods

The experimental procedures used throughout this study were approved (no 32/2010) by the Local Ethics Committee on Animal Experimentation of University of Life Sciences of Lublin, Poland. The chickens were maintained in an animal house according to the guidelines of this committee. The experiment complied with the Guiding Principles for Research Involving Animals.

Animals and experimental design

The study was carried out on 80 healthy male Ross 308 broiler chickens that were randomly assigned to two weight-matched groups after hatching, each in one repetition of 40 chicks. At the age of 1 day, the chickens were assigned to a control group (40 birds were divided into 10 pens with 4 birds in each pen) and a group fed with lowered level of organic zinc as glycinate chelate as an experimental group (40 birds were divided into 10 pens with 4 birds in each pen). The animals were reared on a straw mulch and kept under standard rearing conditions and air temperature set at the optimal level depending on age. During the first week, the chickens were kept at 33°C, which was reduced by 2°C weekly, until the final temperature of 24°C. The chickens had constant access to fresh water, appropriate feed supplied *ad libitum* in accordance with this stage of the production cycle (Table 1). The chickens received a starter diet in the form of crumble, and grower and finisher diets in the pellet form. The birds were fed with a diet corresponding to periods of rearing: starter (1–21 days), grower (22–35 days), and finisher (36–42 days). At the end of the experiment, 10 birds randomly selected from the control (1 bird from each pen) and experimental (1 bird from each pen) groups were slaughtered at the age of 42 days. Ten hours before the slaughter, the selected birds were not given feed, but only provided with unlimited access to water. The male Ross broiler chickens were weighed after hatching and before the slaughter.

Daily body weight gain, feed intake for the whole period, and FCR (feed conversion ratio as a measure of how well a flock converts feed intake (feed usage) into live weight) were calculated.

Table 1. Composition and nutritive value of the experimental diet

Ingredient (%)	Starter (1–21 day)	Grower (22–35 day)	Finisher (36–42 day)
Corn	24.5	40.0	40.0
Wheat	42.9	27.9	28.8
Soybean meal *	25.0	24.9	22.9
Soybean oil	2.50	3.69	3.98
Phosphate 1-Ca	0.90	0.90	0.81
Ground limestone	1.40	1.13	1.09
Sodium bicarbonate	0.08	0.08	0.08
NaCl	0.29	0.25	0.26
Vitamin premix (no Zn)	0.50 a	0.50 b	0.50 c
Protein-fat concentrate **	1.00	-	1.00
DL-methionine 99%	0.30	0.23	0.23
L-lysine HCl	0.42	0.28	0.27
L-threonine 99%	0.18	0.13	0.07
The nutritional value of 1 kg diet:			
[e] ME (MJ kg^{-1})	12.8	13.2	13.3
[d] Total protein (%)	21.2	20.4	19.9
[d] Crude fibre (%)	1.64	1.59	1.73
[d] Crude fat (%)	4.57	5.42	5.53
[d] Lysine (%)	1.28	1.14	1.08
[d] Met + Cys (%)	0.92	0.81	0.82
[d] Total Ca (%)	0.87	0.79	0.76
[d] Total P (%)	0.65	0.66	0.64
[e] Bioavailable P (%)	0.42	0.41	0.39
[e] Total Ca/P bioavailable	2.12	1.90	1.92
[d] Zn from plants in basal diet (mg)	28.3	25.9	24.9
[d] Fe (mg)	40.2	39.9	39.7
[d] Cu (mg)	14.5	14.7	13.6

[a] Content of vitamins and minerals per kg of Starter: Mn 100 mg, I 1 mg, Fe 40 mg, Cu 16 mg, Se 0.15 mg, vitamin A 15 000 IU, vitamin D_3 5 000 IU, vitamin E 75 mg, vitamin K_3 4 mg, vitamin B_1 3 mg, vitamin B_2 8 mg, vitamin B_6 5 mg, vitamin B_{12} 0,016 mg, biotin 0.2 mg, folic acid 2 mg, nicotinic acid 60 mg, pantothenic acid 18 mg, choline 1 800 mg.

[b] Content of vitamins and minerals per kg of Grower: Mn 100 mg, I 1 mg, Fe 40 mg, Cu 16 mg, Se 0,15 mg, vitamin A 12 000 IU, vitamin D_3 5 000 IU, vitamin E 50 mg, vitamin K_3 3 mg, vitamin B_1 2 mg, vitamin B_2 6 mg, vitamin B_6 4 mg, vitamin B_{12} 0.016 µg, biotin 0.2 mg, folic acid 1.75 mg, nicotinic acid 60 mg, pantothenic acid 18 mg, choline 1 600 mg.

[c] Content of vitamins and minerals per kg of Finisher: Mn 100 mg, I 1 mg, Fe 40 mg, Cu 16 mg, Se 0.15 mg, vitamin A 12 000 IU, vitamin D_3 5 000 IU, vitamin E 50 mg, vitamin K_3 2 mg, vitamin B_1 2 mg, vitamin B_2 5 mg, vitamin B_6 3 mg, vitamin B_{12} 0.011 µg, biotin 0,05 mg, folic acid 1.5 mg, nicotinic acid 35 mg, pantothenic acid 18 mg, choline 1 600 mg.

[d] Analysed values.

[e] Calculated values.

* 46% total protein in dry matter.

** 1 kg protein-fat concentrate contains: 2% crude fat, 39% crude protein, 10.8 MJ ME.

Supplementation of Zn amino acid chelate

The basal diet was prepared on the basis of maize and wheat middling as well as soybean meal to contain adequate levels of all the nutrients as recommended except for Zn. The control group (CONT) was fed with basal diet supplemented with the premix, which provided the recommended Zn level for Ross 308 in the inorganic form (Zn as zinc oxide in 100% of the total requirement of the component recommended for Ross 308 broiler chicks). The experimental group (OG25) was fed with basal diet and the premix, which provided Zn in the organic form as glycine chelate (Gly-Zn) in the amount of 25% of the total daily recommended amount for Ross 308 broiler (zinc was added to the premix (no Zn) at the dose of 25 $mg \cdot kg^{-1}$). The experiment involved the use of Glystar Forte chelate (Arkop Sp. z o.o., Bukowno, Poland) containing 16% of Zn and zinc oxide containing 78% of Zn, which was added to the premix (no Zn) at the dose of 100 $mg \times kg^{-1}$ to chickens from the control groups. The basal corn-soybean meal diets (Table 1) containing 24.99÷28.32 $mg \times kg^{-1}$ of Zn as the feed basis (by analysis) were formulated to meet or exceed nutritional requirements. The amount of Zn in the diet was based on nutritional recommendations for Ross 308 broilers (Aviagen, 2014), i.e. 100 $mg \cdot kg^{-1}$ of Zn, irrespective of its content in the components of the basal diet. According to these recommendations, the Zn content should be the same in all periods of rearing, which was taken into account in the study (Aviagen, 2013; Cao et al., 2002; NRC, 1994). Earlier recommended amount of Zn was 14–57 $mg \cdot kg^{-1}$ for different market broilers except for growing male Ross 308 chickens (NRC, 1994). An application of the various chelates has been regulated by the EU Directive 1334/2003, in which the intrinsic content of trace elements taking into account all their sources, is expressed in $mg \cdot kg^{-1}$ (EC, 2003).

Bone analysis

Right and left tibiae were isolated, their weight and length were measured after removal of soft tissues. Each bone was wrapped in gauze soaked in isotonic saline and stored at –25ºC for further analysis. Moreover, the weight/length index was calculated as a weight (mg) to the length (mm) ratio. This index shows changes in bone mineralization (Ziaie et al., 2011; Ziaie et al., 2001).

Analysis of mechanical and geometrical properties

Bone mechanical properties were determined identically for two groups after 3-hour thawing at room temperature using the three-point bending test. Bone mechanical properties were examined on a Zwick Z010 universal testing machine (Zwick GmbH & Company KG), equipped with a measuring head (Zwick GmbH & Company KG) of operation range up to 10 kN, linked to a computer with test TestXpert II 3.1 software (Zwick GmbH & Company KG). The distance between supports was set at 40% of total tibia length. The measuring head loaded bone samples with a constant speed of 10 $mm \times min^{-1}$. The maximum elastic strength (Wy) and the ultimate strength (Wf) were determined as described previously (Ferretti et al., 1993; Tomaszewska et al., 2012 a; Tomaszewska et al., 2012 b).

On the basis of measurements of the horizontal and vertical diameters of the mid-diaphyseal cross-section of bone, the cross-sectional area (A), the second moment of inertia (Ix) and the mean relative wall thickness (MRWT) were derived. Moreover, the cortical index (CI) of the bone was estimated as described previously (Ferretti et al., 1993; Tomaszewska et al., 2012 a; Tomaszewska et al., 2012 b).

Histomorphometry

After removal of soft tissues, the joint was opened and full-thickness cylindrical 20 mm thick samples (cartilage and bone) were taken from the same anatomical position in the knee joint i.e. from the middle of the lateral tibial condyle. Sagittal bone sections were cut perpendicular to the articular surface. The tissue samples were subjected to histology and microscopy procedure as described previously (Tomaszewska et al., 2012 a; Tomaszewska et al., 2012 b).

Microscopic bright field images were collected using a confocal microscope Axiovert 200M (Carl Zeiss, Jena, Germany) equipped with a camera AxioCam HRc (Carl Zeiss, Jena, Germany) and a fluorescent lamp (excitation wavelength 450–490 nm).

Among the parameters recommended by the American Society for Bone and Mineral Research (Mocetti et al., 2000; Parfitt et al., 1987), the bone volume (BV) and tissue volume (TV) were measured in photographs of the bone tissue sections using the pixel count, and relative bone volume (BV/TV%) was assessed as described previously (Tomaszewska et al., 2012 a; Tomaszewska et al., 2012 b). Other parameters examined for trabecular bone (epiphysis and metaphysis) included the fractal dimension of the trabecular bone (D) defined as the complexity of the borderline, the mean and maximum trabecular thickness (Tb.Th), the number of osteocytes per square millimeter of trabecula (NOt/mm^{2}Tb), and the trabecular separation (Tb.Sp) defined as the distance between the edges of adjacent trabeculae (measured directly), the number of osseous lacunae per mm^2 of trabeculae. The non mineralized space that osteocyte occupies was defined as an osseous lacuna.

Growth hormone and bone turnover markers measurement

Each chicken was fasted before blood collection. The blood was collected using standard venipuncture, and after clotting was centrifuged and frozen at –80°C for later analysis. All the samples were determined in duplicate.

The serum concentration of chicken growth hormone (GH) was determined with a GH kit (ELISA; Uscn Life Science Inc. Wuhan, China). The sensitivity in this assay was 0.056 ng·mL^{-1}. The serum chicken insulin-like growth factor (IGF-1) concentration was determined with an IGF-1 kit (ELISA; Uscn Life Science Inc. Wuhan, China). The minimum detection was 7.4 pg·mL^{-1}. The serum osteocalcin (OC) concentration was determined with the use of chicken Osteocalcin kit (ELISA; Uscn Life Science Inc. Wuhan, China). Minimum detectable concentration was 0.67 pg·mL^{-1}. The serum leptin (LEP) concentration was determined with the use of chicken Leptin kit (ELISA; Uscn Life Science Inc. Wuhan, China). Minimum detectable concentration was 14.8 pg·mL^{-1}.

Selected micro- and macroelements in the serum

The serum concentration of Zn, Cu, Ca, Mg, Fe, P was determined by a colorimetric method using a Metrolab 2300 GL unit (Metrolab SA, Argentina) and sets produced by BioMaxima (Lublin, Poland).

Zinc analysis in bone

After evaluating the strength and structural properties, the femurs were defatted, dried to constant mass, and finally mineralised in a muffle furnace at 600°C (AOAC, 2000). The content of Zn in bones was determined by the atomic absorption spectrometry using a Unicam 939/959 apparatus. The content of Zn in the bone was calculated as its content in crude ash.

Statistical analysis

The results are expressed as means ±SD (standard deviation). The differences between means were tested with the Student-T test. Normal distribution of data was examined using the W. Shapiro-Wilk test and equality of variance was tested by the Brown-Forsythe test. A $P \leq 0.05$ was considered statistically significant. All statistical analyses were carried out by means of Statistica 8.0 software (StatSoft Inc., Tulsa, OK, USA).

Results

Birds with approximately the same initial weight were used. The average body weight of male Ross broiler chickens after hatching was 48 g ±0.64. At slaughter, birds in the control group weighed about 2600 g ±340 and 2615 g ±273 in the group supplemented with zinc as glycine chelate in 25% of the recommended amount, i.e. there was no difference in the weight.

Daily body weight gain in the control group was 62±5 g, and 61±6 g in the group supplemented with zinc. Feed intake for the whole period was 4351±299 and 4389±150 in the control group and the group supplemented with zinc, respectively. An indicator of management performance such as FCR (for the whole period) reached the value of 1.68±0.11 g/g in the control group and 1.73±0.15 g/g in the group supplemented with zinc. Daily body weight gain, feed intake and FCR did not differ between the groups.

Supplementation of zinc as glycine chelate in 25% of recommended amount to male Ross broiler chickens did not affect morphology, mechanics and geometry of femur when compared with the control group (Table 2).

Analysis of the trabecular bone of tibia is presented in Table 3. Histomorphometrical analysis revealed significantly lower values of the relative bone volume of femur in the birds that received glycine chelate for 42 days of life. The supplementation of organic zinc in 25% of the recommended amount also resulted in reduced mean and maximal trabecular thickness of tibia. Moreover, trabecular space increased in tibia. The supplementation did not influence the complexity of the borderline and the number of osteocytes.

The results of the analyses of the growth hormone, leptin and bone turnover markers are presented in Table 4. It was shown that the organic Zn administration in 25% of the total requirement of the microelement recommended for Ross 308 broiler decreased the concentration of osteocalcin.

Table 2. The mechanical and geometric properties of tibia in male Ross 308 chickens

	CONT (n=10)	OG 25 (n=10)
Bone weight (g)	12.9±2.14	14.4±2.19
Length (mm)	101.9±2.6	99.0±1.2
The weight/length index (mg·mm^{-1})	129.7±28.5	135.6±18.2
Ultimate force (N)	295.0±59	310.0±57
Max. elastic force (N)	191.0±43	160.0±56
A (mm^2)	41.9±3.9	42.0±6.6
MRWT	0.79±0.24	0.67±0.20
IC	42.4±4.6	39.2±7.5
Ix (mm^4)	228.0±58	257.0±34

Data given are Mean ±SD.

* Indicates significant differences between CONT and OG 25 where P≤0.05.

CONT = basal diet with added Zn in the inorganic form; IG25 = basal diet with added Zn in the organic form in 25% of recommended amount; A – cross section area; MRWT – mean relative wall thickness; IC – cortical index; Ix – the second moment of inertia.

Table 3. The trabecular bone morphology of tibia in male Ross 308 chickens

	CONT (n=10)	OG 25 (n=10)
BV/TV(%)	22±4	15±3*
Tb.Th mean (µm)	194±98	75±10*
Tb.Th max (µm)	322±197	151±25*
Tb.Sp (µm)	162±69	295±53*
N.Ot/mm^{2}Tb	2154±439	1713±757
Fractal Dimension	1.45±0.16	1.28±0.03

Data given are Mean ±SD.

* indicates significant differences between CONT and OG 25 where P≤0.05.

CONT = basal diet with added Zn in the inorganic form; IG25 = basal diet with added Zn in the organic form in 25% of recommended amount; BV/TV% – relative bone volume; Tb.Th. – the trabecular thickness; Tb.Sp. – the trabecular separation; N.Ot – the number of osteocytes.

Table 4. The concentration of growth hormone (GH), insulin-like growth factor-1 (IGF-1), osteocalcin (OC) and leptin (LEP) in the blood serum of male Ross 308 chickens

	CONT (n=10)	OG25 (n=10)
GH (ng·mL^{-1})	3.62±1.53	2.91±0.59
IGF 1 (pg·mL^{-1})	145.0±63	156.0±75
OC (pg·mL^{-1})	49.7±5.73	40.1±3.80*
LEP (pg·mL^{-1})	123.0±38	113.0±32

Data given are Mean ±SD.

* Indicates significant differences between CONT and OG 25 where P≤0.05.

CONT = basal diet with added Zn in the inorganic form; IG25 = basal diet with added Zn in the organic form in 25% of recommended amount.

Table 5. The concentration of selected minerals in the blood serum of male Ross 308 chickens

	CONT (n=10)	OG 25 (n=10)
Zn (μmol·L^{-1})	24.5±1.3	25.5±1.6
Cu (μmol·L^{-1})	1.70±0.5	2.20±0.2
Ca (μmol·L^{-1})	2.30±0.1	2.20±0.1
Mg (μmol·L^{-1})	0.80±0.2	0.80±0.1
Fe (μmol·L^{-1})	8.90±1.1	8.50±1.2
P (μmol·L^{-1})	2.10±0.4	1.90±0.1

Data given are Mean ±SD.

* Indicates significant differences between CONT and OG 25 where P≤0.05.

CONT = basal diet with added Zn in the inorganic form; IG25 = basal diet with added Zn in the organic form in 25% of recommended amount.

The results of selected macro- and microelements analyses are presented in Table 5. It was shown that Zn administration as glycine chelate in male broiler chickens did not influence their concentration.

The contents of bone zinc detected in the controls and the group supplemented with the organic source did not differ. The amount of zinc in the bones of the control chickens reached the value of 484±17 mg·kg^{-1}, while in the group supplemented with organic Zn, it reached a value of 489±26 mg·kg^{-1}.

Discussion

Zinc is important for a variety of physiological processes essential for optimal bird growth and development. It plays a crucial role as a catalyst in many enzymes and hormones influencing bone development. In the skeleton, zinc is an important mineral source for metabolic needs (Sahraei et al., 2012). In poultry, zinc deficiency results in reduction of weight gain, skeleton malformation, and insufficient bone mineralization (Sahraei et al., 2012). Because of the most dynamic growth rate in broilers and more frequently occurring disorders such as tibial dyschondroplasia or chondrodystrophy, proper bone development, especially in pelvic limbs, is very important for proper locomotor function in chickens.

The results of this study are new and, to our knowledge, the organic source of zinc (glycine chelate) administered below the recommended level has not been investigated so far in any other animals in relation to bone tissue histomorphometry, geometry, and mechanics as well as somatotropic axis (growth hormone and IGF 1), bone turnover markers, and zinc bone content. The results of this study showed that zinc lowered to 25% of recommended amount in Zn-glycine chelate form did not affect daily body weight gain, feed intake, FCR, and the weight and length of leg bone.

This result is in agreement with other studies, which showed that the tibia weight in broiler Ross chickens did not change when the basal diet was supplemented with zinc in the amount of 50 mg·kg^{-1} as an organic form. Moreover, contrary to our study, this experimental diet contains higher amount of zinc and also manganese and copper and was given to female chickens from the 11th to 35th day of the grower stage (El-Husseiny et al., 2012).

Our male birds were fed with a diet supplemented with 25% of the daily recommended amount of zinc. This study showed that supplementation with trace minerals in the amount below the daily recommendation did not inhibit weight gain in the male Ross broiler chickens, as the final body weights of the birds from the group receiving a diet reduced to a one fourth of the daily Zn recommendation as chelate or basal diet with zinc oxide (containing Zn in 100% of the total requirement of the component recommended for Ross 308 broiler chicks) did not differ between each other. Probably, the diet with 25% of the daily recommended amount of zinc sufficiently supported growth and allowed reaching optimal body weight. Perhaps the experimental period was too short to trigger greater inhibition of general growth. But, this period was in agreement with other study (Ziaie et al., 2011) and even longer than that lasting from the 11th to 35th day of age (El-Husseiny et al., 2012).

Investigation of bone development in relation to trace minerals from organic sources in poultry is very important because bone weakness and lesions lead to decreased meat production. Weak legs are associated with reduced feed intake and finally affect weight gain. The bone leg quality is commonly used as an indicator of mineral adequacy in poultry diet (Sahraei et al., 2012).

The zinc bone content in our birds from the group supplemented with a lower level than the recommended one did not differ from the content in bone from the control group fed with the diet supplemented with zinc oxide. The diet with the organic zinc administered at the lowered level than recommended did not reduce bone mineral density, compared to the control diet. In our birds, no change was observed in the weight/length index. The weight/length index is a simple index of bone density introduced for the first time by Seedor et al. (1991). In general, the higher the bone weight/length index, the denser is the bone (Seedor et al., 1991; Ziaie et al., 2011).

Zinc plays an important role in cell proliferation and differentiation and affects cellular activities through hormones and growth factors. Zinc deficiency reduces the serum concentration of IGF-1 (Wang et al., 2002). Cellular changes are observed locally in an area remote from blood vessels, where zinc bioavailability from blood supply is reduced (Wang et al., 2002). For this reason, it is suggested that longitudinal growth might be very sensitive to zinc status. The tissue mineral concentration is an indicator of body storage and mineral status and can be used as a biomarker of its bioavailability. Moreover, a reduction in the dietary supply of minerals involves a combination of responses including reduced exertion (Skřivan et al., 2005; Wang et al., 2002).

In our male Ross broiler chickens, no difference was observed in the concentrations of zinc, growth hormone, and other factor involved in the general growth like IGF-1. Probably, for this reason the optimal growth of the birds was supported. However, the presented birds showed a reduced concentration of osteocalcin, a typical non-collagenous bone protein that reflects bone formation. Our observation showed that the low concentration of zinc in poultry diet had adverse effects. Histomorphometric analysis showed rapid loss in real bone volume in the trabecular bone of the leg in birds fed with the organic source of zinc in 25% of the recommended amount.

This treatment also resulted in a decrease in the thickness of trabeculae and an increase in the trabecular space. This result might suggest that there was insufficient bone formation or bone loss, and the trabeculae looked like osteoporotic. This corresponded to the reduced osteocalcin concentration.

Zinc deficiency in chickens is characterized by leg abnormalities (Starcher et al., 1980). These differences are determined by the growth rate (Srinivasan et al., 2000; Webster, 2004; Whitehead, 2004). Bent lower legs are more common in cocks than in hens and occur in young animals (Paz et al., 2009; Paz et al., 2013). Moreover, tibial dyschondroplasia is one of the most common skeletal lesions that results in bone deformation (Śliwa et al., 1996).

In the present study, zinc was supplied not in the recommended dose but even in an amount lowered to one fourth thereof, which did not influence mechanical parameters. However, it seems that there was a strong tendency towards a reduced value of the maximum elastic force as compared to the control diet containing zinc oxide in 100% of the recommended amount. Probably, this organic supplementation of zinc in 25% of the recommended amount did not support the optimal bone development of growing male broiler chickens through its natural high bioavailability. This amount was insufficient for the birds.

This finding is not in agreement with a study that reports improved tibia strength by supplementation of female broilers with low-zinc diet (in the amount of 50 $mg \cdot kg^{-1}$ as an organic form), probably because our birds received zinc in the level of 25% of the recommended amount (El-Husseiny et al., 2012).

In conclusion, this study showed that the dietary treatment with organic zinc in 25% of the recommended amount did not affect the general growth of the birds, as indicated by the concentrations of growth hormone, IGF-1, and leptin. Moreover, the use of chelate in the amount limited to 25 $mg \cdot kg^{-1}$ did not result in worse morphologic, geometric, and mechanical parameters of chicken tibia compared to the recommended dose (100 $mg \cdot kg^{-1}$). However, histomorphometry revealed a disproportionately large osteoporotic surface. The changes in tibial trabecular bone suggest that the diet supplemented with zinc as glycinate chelate only in 25% of the total requirement of the component recommended for Ross 308 broiler chicks seems to be insufficient for tibia development. This finding might suggest a necessity of supplementation with higher levels of trace minerals when added as glycine chelate.

Conflict of Interest

The authors declare no conflict of interest.

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