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THE INFLUENCE OF DIETARY ZINC CONTENT ON MECHANICAL PROPERTIES OF CHICKEN TIBIOTARSAL BONE

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The authors evaluated the effect of diets with different Zn levels on mechanical properties of bones in 60 Ross 308 hybrid broilers, from 2 to 35 days of age, which were randomly divided in 2 groups, with 30 chickens each. The first group (Zn50) was fed a commercial basal diet with no Zn additives (zinc content - 50 mg/kg feed). The second group (Zn100) was fed the basal diet + 50 mg of Zn/kg feed. In this group the dietary Zn level was increased by adding 62.23 mg of zinc oxide (ZnO)/kg feed to starter and grower feeds. In group Zn100 compared to Zn50 the values of blood zinc level were significantly higher on days 7 and 35 ($p<0.01$ and $p<0.05$). Significant differences in bone zinc content were not detected. On days 7, 21, and 35 the selected mechanical properties of the tibiotarsus were evaluated. The results obtained in the experiment showed differences in biomechanical competence of broiler bones from group Zn50 compared to Zn100. In broilers from group Zn100, there were significantly higher values in the limit of elasticity (Re), bending strength (Rm) and fracture stress (Rl) ($p<0.05$ to $p<0.01$) of chicken bones on day 35. The mean levels (117.80 ± 19.66, 126.70 ± 32.56, 114.10 ± 19.92, respectively) were determined in broiler bones from group Zn100 compared to Zn50 (95.27 ±18.71, 100.2 ± 20.56, 80.93 ± 35.22, respectively). In group Zn100 were observed significantly increased values of the bone cross-section area (Ar) on day 35 and moment of inertia (I) on days 21 and 35 ($p<0.05$). The clinical examination of the locomotory system showed bone deformities of the intertarsal joint in 10% of broilers from group Zn50. Present results suggest that the determination of the weight bearing of the tibial bone evaluated by assessing of mechanical properties of bones is an indicator of the locomotory system disorders and the Zn content in feed has a direct and statistically significant effect on bone strength in broiler chickens.

Key words: bone, broiler, mechanical properties, three point bending test, zinc

INTRODUCTION

Orthopaedic-related leg problems contribute to a large percentage of profit loss in the poultry industry (Rath *et al*., 2000). Fast growing hybrids might have different requirements on minerals and feed composition (Sesztáková *et al*., 2010). There is a need for better understanding of bone strength in poultry because bone breakage and associated infections contribute to mortality, low productivity, and carcass condemnations. The economic costs associated with bone problems in poultry can add up to several hundred million dollars a year (Rath *et al*., 2000). Bone status is commonly used as an indicator of mineral adequacy in poultry diets (Rath *et al*., 1999). The extent of bone mineralization affects bone strength (Reichmann and Connor, 1977), and poor mineralization has been associated with increased risk of fractures (Molnár, 2010; Blake and Fogelman, 2002). Weak bones result in breakage during processing and lower meat grade. Also, weak legs often result in reduced feed intake thus affecting the weight gain, as well as the quality and number of eggs laid (Orban *et al*., 1999). It has been recognised that different compositions of diet affect considerably the growth, mineral composition and structure of bones. This in turn affects their mechanical properties and may produce conditions for development of various osteopathies. The study of mechanical properties of bones allows one to explain new relationships and mechanisms of development of relevant diseases (Baranová *et al*., 2008). Several studies dealing with biochemical changes in bones resulting from zinc deficiency suggest the essentiality of zinc for bone structure. Abnormal bone development is one of the primary symptoms associated with zinc deficiency in birds (Jurajda, 2003). Scrimgeour *et al.* (2007) and Cowin (2001) reviewed the influence of dietary zinc on the bone integrity and mechanical properties of bones and described the effect of zinc intake on the skeletal system due to changes of biomechanical competency of bone tissue and decreased density of bones.

The available literature provides different data related to optimum content of zinc in poultry feed. The daily zinc requirements depend on species, age and productivity of poultry. The range is in the interval of 30-60 mg/kg of feed (Jantošovič *et al*., 1998). Other authors reported that the optimum Zn range for broilers is 40–70 mg of Zn/kg feed (Underwood and Suttle, 1999) or up to 68 mg of Zn/kg feed (Wang *et al*., 2002). The above data indicate that a relatively wide range of Zn content in food may affect the bone strength. An advised content of Zn for Ross broiler chickens in complete diet according to The Ross 308 Manual (2007) is 100 mg/kg of feed, but despite of this many standard commercial poultry diets do not contain the given level.

The aim of this study was to investigate the influence of different zinc levels in the diet on blood and bone zinc content and direct mechanical characteristics of the tibial bone and assess the value of fractography results to determine the weight bearing property of tibial bone as an indicator of locomotory system disorders in broiler chickens.

MATERIALS AND METHODS

The study was carried out on 60 two days old chickens of Ross 308 hybrid without segregation according to sex. All birds were placed into 10 m^2 pens with wood shavings at a density of 10 birds/m^2 in a continuously illuminated room. The temperature in the room was initially maintained at 33°C and was reduced by 3°C/wk until it reached 21°C which was maintained until the end of the experiment. All birds were divided into 2 groups; 30 chickens in each. All birds were fed diets with identical nutritional density and water was offered *ad libitum*. The feed provided to broilers corresponded to two phases: starter diet (1 - 14 days) and grower diet (15 - 35 days). The ingredients and nutrient composition of broiler starter and grower diets are presented in Table 1. The first group (Zn50) was fed commercial basal diet with no Zn additives (zinc content - 50 mg/kg feed). The second group (Zn100) was fed basal diet + 50 mg of Zn/kg feed. In this group the dietary Zn level was increased by adding 62.23 mg of zinc oxide (ZnO)/kg feed. Both groups were fed their diets *ad libitum* until day 35 of age. On days 7, 21 and 35, blood samples were collected from the *jugular vein* of 10 broilers from each group. The samples were analysed spectrophotometrically for blood Zn level using the test by Randox Laboratories LTD (Ardmore, UK) and a UV-VIS Shimadzu UV-1601 spectrophotometer (Japan).

Table 1. Composition of the control broiler diet

Ingredient	Starter	Grower
Nitrogen substances, g/kg	200.0	180.0
Metabolizable energy, MJ/kg	12.0	2.0
Ash, g/kg	70.0	70.0
Fibre, g/kg	35.0	40.0
Methionine + cystine, g/kg	7.5	7.5
Methionine, g/kg	4.5	4.0
Lysine, g/kg	11.0	9.5
Ca, g/kg	8-14.0	8-14.0
P, g/kg	5.0	5.0
Na, g/kg	1,2-4.0	1.2-4.0
Mn, mg/kg	60.0	60.0
Fe, mg/kg	60.0	60.0
Cu, mg/kg	4.0	4.0
Zn, mg/kg	50.0	50.0
Vit. A, IU/kg	10 000	8 000
Vit. D3, IU/kg	2 000	800
Vit. E, IU/kg	10.0	10.0
Vit. B2, mg/kg	4.0	3.0
Vit. B12, mcg/kg	20.0	20.0

On days 7, 21 and 35, the locomotory system of 10 broilers from each group was examined to find the skeletal disorders and then broilers were euthanized by cervical dislocation. The tibiotarsal bones from both legs were dissected from fresh carcasses and stripped of soft tissues. They were stored in plastic bags at –20^{o}C and warmed up to 20^{o}C before testing. The samples of the left chicken tibiotarsal bones were digested in microwave oven MLS 1,200 MEGA (Milestone) using 5 m/L HNO_3 and 1 mL HCl per gram of sample. The digested samples were analyzed for the presence of Zn by using an atomic absorption spectrophotometer (AAS), Unicam Solar 939. The elements were measured under optimised operating conditions by AAS with an air–acetylene flame. The flame conditions were those recommended by the instrument manufacturer for zinc (wavelength 213.9 nm, band pass 0.5 nm).

Static tests of mechanical properties of the right tibiotarsal bones were carried out on a universal tensile testing machine FP100/1 using a three point bending test at ambient temperature 20^{o}C (Baranová, 2008). The rate of loading was 2.5 mm.min^{-1}, the loading force ranging from 1 kN to 40 N and the distance of supports from 25 to 60 mm, according to the size of bones. The bones were placed on supports in such a way so that the plane of the smaller dimension of the bone was parallel with the loading force. Deflection of bones was scanned for each bone separately and registered with 10-fold magnification (Figure 1). The force-deformation diagram was used to evaluate the following parameters: forces

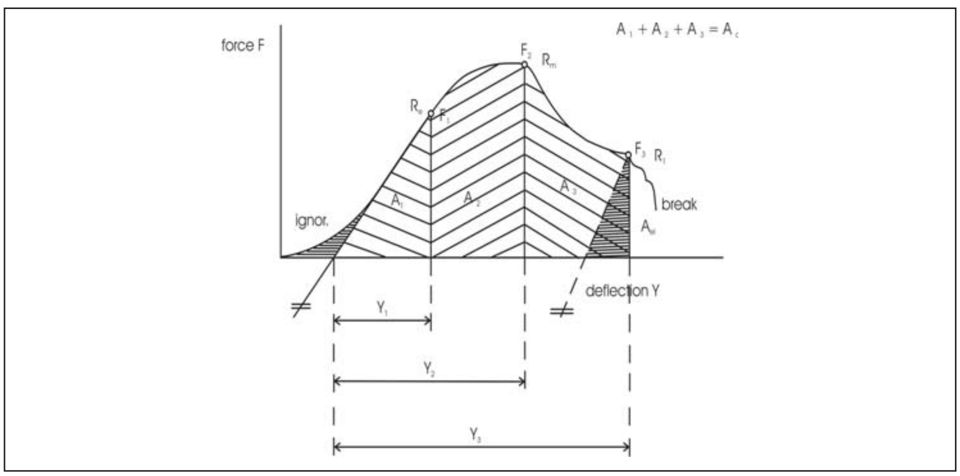

Figure 1. The bone force-deflection curve with indication of respective parameters: Re (limit of elasticity), Rm (bending strength), Rl (fracture stress), F1 (force at the limit of elasticity), F2 (maximum loading force), F3 (force in the moment of bone breaking), Y1 (bone deflection corresponding to force F1), Y2 (bone deflection corresponding to force F2), Y3 (bone deflection corresponding to force F3), A1 (area below the force deflection curve, starting from the beginning of loading up to force F1), A2 (area below the force deflection curve, between the forces F1 and F2), A3 (area below the force deflection curve, between the forces F2 and F3), Ac (total work expended on deformation and failure), Ael (elastic energy released at fracture)

F1, F2, F3; bone deflections Y1, Y2, Y3; areas A1, A2, A3. The obtained values and relevant formulas allowed us to calculate the respective properties of bones (Table 2). The parameters of bones "D" (external bone diameter in the plane parallel to the loading force), "B" (external bone diameter in the plane perpendicular to the loading force), "d" (internal bone diameter in the plane parallel to the loading force) and "b" (internal bone diameter in the plane perpendicular to the loading force) were measured with 0.01 mm accuracy using a technical slide calliper. Statistical data analyses were conducted using the statistical program GraphPad Prism5. The data were subjected to correlation analysis to reveal potential relationships between them. Student's *t*-test was applied to determine significance of differences between groups Zn50 and Zn100. The 0.05, 0.01 and 0.001 levels of significance were used.

Table 2. Formulas for calculation of relevant parameters (Baranová, 2008)

Mechanical properties - parameters	Characteristics	Formula for calculation
Moment of inertia (I)	The geometrical characteristic of a cross-section which allows one to calculate the minimum bending stress in the most stressed cross-section of the bone	I (mm^4) = ($B.D^3 - b.d^3$) . $\pi/64$ D, B, d, b = external and internal bone diameters
Bone cross-section area (Ar)	The bone cross-section area at the point of fracture	Ar (mm^2) = (B.D – b.d) . $\pi/4$
Limit of elasticity (Re)	The stress up to which the bone is deformed elastically (reversibly)	Re (Mpa) = F.s.D / (8.I) F1= force at the limit of elasticity s = distance between supports group 1 (7d old) = 25 mm group 2 (21d old) = 40 mm groups 3 (35d old) = 60 mm
Modulus of elasticity (E)	Constant of proportionality between stress σ and deformation ε	E (Mpa) = F1.s^3 / (48.I.Y1) Y1 = bone deflection corresponding to force F1
Bending strength (Rm)	The highest bending stress on the surface of the flexed bone	Rm (Mpa) = F2.s.D / (8.I) F2 = maximum loading force
Fracture stress (Rl)	The bending stress at the point of fracture	Rl (Mpa) = F3.s.D / (8.I) F3 = force in the moment of bone breaking
Toughness (Huz)	The work needed to deform the bone up to its failure	Huz (N/mm = 10^{-3} J/mm^2) = (Ac – Ael)/Ar Ac = total work Ael = elastic energy

RESULTS AND DISCUSSION

Zinc status: Plasma or serum zinc is currently the most widely used and accepted biomarker of zinc status despite poor sensitivity and imperfect specificity (Sian *et al.*, 1996). The zinc blood levels should be evaluated carefully because changes in blood level of zinc could be detected only after long-term deficiency or abundance in the diet. The blood zinc level decrease is often observed only after growth retardation (Underwood and Suttle, 1999). The concentration of zinc in tibiotarsal bone and plasma of broilers increases linearly with the concentration of Zn in broiler feed (Mohanna and Nys, 1999). Hill *et al*. (2000) described that the structural and functional changes due to zinc deficiency in bone tissue structure are associated with a wide range of biochemical changes in the blood and tissues. As a first sign there is a decrease in Zn concentration in plasma or serum. In our experiment, a lower blood zinc level was observed from day 7 until the end of the experiment in group fed the basal diet (50 mg/kg of feed) compared to the birds fed diets with an increased amount of Zn (100 mg/kg of feed) (Table 3). Significant differences between group Zn50 and Zn100 were recorded on day 7 (p<0.01) and 35 (p<0.05). Serum Zn levels measured in our experiment correlated with the reference range for serum Zn level in broiler chickens. According to Jongbloed *et al*. (2002) bone and pancreatic zinc are the best response criteria to assess the biological value of zinc sources in monogastrics. Underwood and Suttle (1999) found a gradual decrease in Zn concentration in wool, feather and bones caused by lower zinc content in the feed. Scrimgeour *et al*. (2007) found a positive correlation between dietary and bone zinc level, Mohanna and Nys (1999) recorded that tibia zinc content increases linearly with dietary zinc level, which does not agree with the result in this experiment. In groups Zn50 and Zn100 the significant differences in bone zinc content were not detected (Table 3).

Table 3. Comparison of serum and bone Zn levels in broiler chickens between group Zn50 and Zn100 at the age of 7, 21 and 35 days

Blood serum (μmol.l^{-1})		7 d	21 d	35 d	Tibia (mg/kg)		7 d	21 d	35 d
Zn50	Zn	28.78	33.53	28.08	Zn50	Zn	151.80	152.00	232.20
	SD	3.78	7.04	4.65		SD	15.32	12.48	38.58
	N	10	10	10		n	10	10	10
Zn100	Zn	36.06**	36.44	36.75*	Zn100	Zn	143.00	159.00	197.80
	SD	5.94	11.88	5.82		SD	55.26	16.51	18.97
	N	10	10	10		n	10	10	10

[1]n = number of values (n=10); [2]SD = Standard deviation; *p<0.05; **p<0.01

Mechanical properties of bones: The tibiotarsal bone was selected as an experimental object to determine the selected mechanical properties of chicken bones. According to Shelton and Southern (2007) the breaking strength of a tibial bone was significantly increased in 14 days old broilers fed the diet with supplemental Zn (75 ppm). Scrimgeour *et al.* (2007) claimed that the tibiotarsal bone compared to the femoral bone is more sensitive to different Zn levels. In this study the bending strength (Rm), limit of elasticity (Re) and fracture stress (Rl) were changed significantly in Zn100 which agrees with the results of Shelton and Southern (2007). During the first 3 weeks of the experiment the Re values in groups Zn50 and Zn100 persisted at approximately the same level (Table 4). On day 35 of the experiment in group Zn100 we observed a gradual increase in Re, the difference was statistically significant ($p<0.01$). In group Zn100, the same tendency was observed for Rm and Rl with the maximum values on day 35 ($p<0.05$). The changes in the skeletal system of broilers can be due to culminant ossification of tibiotarsus in this particular phase of the experiment. According to Adamec (2011) the epiphyseal ossification center in the proximal end of the tibiotarsus is radiografically noticeable at the age of 35 days. Consequently, in the last phase of the experiment, the chickens fed increased level of zinc showed a noticeable increase in the level of stress up to which the bone is deformed elastically (reversibly) and after unloading returns to its original shape and dimensions (=Re) and also in the values of the highest bending stress on the surface of the flexed bone (=Rm) and the values of the bending stress at the point of fracture (=Rl). Our results agree with those of Shelton and Southern (2007) and Scrimgeour *et al*. (2007) who reported an influence of Zn on the mechanical properties of bones. These authors described that in animals lower zinc content in feed caused the reduction of bone integrity, bone density, reduction of bone length, deterioration of compact bone formation, changes in biomechanical competency of bone tissue and decrease in density of bones as a result of reduced activity of thin growth bone disc. Many studies were performed to determine the influence of dietary Zn on the integrity and the mechanical properties of the bone. The essential effect of Zn on the bone tissue and bone strength was described by Shelton and Southern (2007), Wang *et al*. (2002), Nielsen *et al*. (1970), Rossi *et al*. (1999) and Rossi *et al*. (2001). Our experiment showed also significant differences in the bone cross-section area at the point of fracture (Ar) which increased linearly in correlation with age, which agrees with the results of Baranová (2001). A significant increase in the values of Ar was detected in group Zn100 on day 35 ($p<0.05$). The moment of inertia (I) increased gradually with age. Baranová (2001) describes that I, as a very sensitive mechanical property of bone, is markedly influenced by the changes in the bone structure. In this experiment the level of I was higher in group Zn100 throughout the experiment except for its first phase (day 7) when an opposite effect was observed. The differences between groups Zn50 and Zn100 were significant on days 21 and 35 ($p<0.05$). No significant differences between groups Zn50 and Zn100 were observed in the modulus of elasticity (E) and toughness (Huz) of bones. The level of E was slightly higher in the bones excised on days 7 and 21 compared to bones excised on day 35. The values of Huz were almost the same in both groups with the exception of day 35. Baranová (2001) examined the mechanical properties of bones in different phases of the age and described that the bones in older animals (at the age of 35 days) tended to express a wider range of measured values than small broiler bones (at the age 7 and 21), which agrees with the results in this experiment.

Table 4. Comparison of the limit of elasticity (Re), bending strength (Rm), fracture stress (Rl), toughness (Huz), modulus of elasticity (E), bone cross-section area (Ar) and moment of inertia (I) between control (C) and experimental (E) broiler chickens at the age of 7, 21 and 35 days

Zn50				Zn100			
	7 d	21 d	35 d		7 d	21 d	35 d
Re (MPa)	61.00	58.60	94.27	Re (MPa)	64.00	59.85	117.80**
SD	10.04	11.12	18.71	SD	7.98	15.80	19.66
Rm (MPa)	73.82	77.60	100.2	Rm (MPa)	76.68	84.09	126.70*
SD	9.89	13.27	20.56	SD	9.09	18.41	32.56
Rl (Mpa)	42.64	51.78	80.93	Rl (Mpa)	42.88	61.46	114.10*
SD	12.65	19.98	35.22	SD	10.23	24.33	19.92
Huz (N/mm)	13.35	10.81	8.95	Huz (N/mm)	13.14	9.26	6.27
SD	2.72	5.11	7.26	SD	4.76	4.42	3.84
E (MPa)	3608	3036	2500	E (MPa)	3469	2624	2785
SD	1329.00	891.00	578.50	SD	630.90	826.10	752.80
Ar (mm^2)	3.47	11.18	24.87	Ar (mm^2)	3.18	12.77	30.48*
SD	0.78	2.04	6.19	SD	0.52	3.06	4.47
I (mm^4)	1.45	16.03	88.47	I (mm^4)	1.30	20.46*	120.70*
SD	0.40	4.11	35.49	SD	0.34	7.57	30.37
n[1]	10	10	10	n[1]	10	10	10

[1]n= number of values (n=10); [2]SD = Standard deviation; *$p<0.05$; **$p<0.01$

Locomotory system: In 3 broiler chickens from group Zn50 the examination of the locomotory system showed various deformities in the last phase of the experiment. In group Zn100 the bone deformities were not detected. Skeletal problems in low zinc diet are recorded by many authors (Jurajda, 2003; Herenda and Franco, 1996; Klimeš, 1970).

The present experiment confirmed the differences in bone biomechanical competence in broilers fed diet with different zinc levels. There were significant changes in mechanical properties of bones suggesting reduced weight bearing capabilities in broilers fed the diet with zinc content 50 mg/kg of feed compared to chickens fed increased levels of zinc (100 mg/kg of feed). The significant reduction in tibial strength and elasticity, the incidence of varus deformities in the 10% of broilers and the significantly lower blood zinc level on day 7 and 35 was observed in the group with lower Zn content. This experiment showed that the determination of the weight bearing of tibial bone evaluated by assessing of its mechanical properties is an indicator of the locomotory system disorders and the

Zn content in the feed has a direct statistically significant effect on bone strength in broiler chickens. The results suggest benefitial effect of zinc supplementation on reducing of locomotory disorders in broilers.

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UTICAJ CINKA U HRANI NA MEHANIČKA SVOJSTVA TIBIO-TARZALNE KOSTI PILIĆA

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SADRŽAJ

U ovoj studiji je procenjivan efekat obroka sa različitim nivoom cinka na mehanička svojstva kostiju kod 60 brojlera hibrida Ros 308, od 2 do 35 dana starosti, podeljenih metodom slučajnog izbora u dve grupe od po 30 pilića u svakoj. Prva grupa (Zn50) je bila na komercijalnoj osnovnoj dijeti koja nije sadržala aditive sa cinkom (sadržaj cinka – 50 mg/kg stočne hrane). Druga grupa (Zn100) je dobijala još 50 mg Zn/kg stočne hrane. U ovoj grupi je povećan nivo cinka dodavanjem 62,23 mg ZnO / kg stočne hrane u starter i grover.

U grupi Zn100 je, u odnosu na Zn50 grupu, koncentracija cinka u krvi bila značajno viša 7-og i 35-og dana ispitivanja (p<0,01 i p<0,05). Nisu otkrivene značajne razlike u sadržaju cinka u kostima. Odabrana mehanička svojstva tibiotarzusa su procenjivana 7-og, 21-og i 35-og dana ispitivanja. Rezultati dobijeni u ovom eksperimentu su ukazali na razlike u biomehaničkim svojstvima kostiju

brojlera iz grupe Zn50 u odnosu na grupu Zn100. Kod brojlera iz grupe Zn100 zabeležene su značajno više vrednosti granice elastičnosti (Re), snage savijanja (Rm) i pritiska preloma (Rl) ($p<0,05$ do $p<0,01$) kostiju pilića i to 35-og dana. Ispitivanjem kostiju brojlera iz grupe Zn100 su utvrđene sledeće srednje vrednosti ovih parametara: 117,80 ± 19,66, 126.70 ± 32,56 i 114,10 ± 19,92, respektikvno. U grupi Zn50 registrovane su sledeće vrednosti: 95,27 ± 18,71, 100,2 ± 20,56 i 80,93 ± 35,22, respektivno.

U grupi Zn100 su zapažene povišene vrednosti zone preseka kosti (Ar) 35-og dana i momenta inercije (I) 21-og i 35-og dana ($p<0,05$). Kliničkim ispitivanjem lokomotornog sistema zapaženi su različiti deformiteti intertarzalnog zgloba kod 10% brojlera iz grupe Zn50. Opterećenje koje može da izdrži tibijalna kost dobijeno je analizom mehaničkih svojstava kostiju. Postignuti rezultati su ukazali da je snaga tibijalne kosti indikator poremećaja funkcije lokomotornog aparata, a sadržaj cinka u stočnoj hrani statistički značajno utiče na jačinu kostiju brojlera.