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Full Length Research Paper

The mechanical properties of broiler chicken bones affected by different dietary zinc levels

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The authors evaluated the effect of diet with different Zn levels on mechanical properties of bones in 120 broilers of Ross 308 hybrid, from 2 to 42 days of age, which were randomly divided into 2 groups, with 60 chickens each. 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 to starter, grower and finisher. The clinical examination of the locomotory system showed the varus bone deformities of the intertarsal joint in 10% of broilers from group Zn50. Both tibiotarsal bones from 10 chickens from each group were dissected from fresh carcasses in weekly intervals on days 7, 14, 21, 28, 35 and 42 to evaluate the strength of tibiotarsus. The results obtained in the experiment showed the differences in biomechanical competence of broiler bones from group Zn50 as compared to Zn100. In broilers from group Zn100, there were significant 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 as compared to Zn50 (95.27 ± 18.71, 100.2 ± 20.56, 80.93 ± 35.22, respectively). Moreover, there was a significant difference in the values of Rl between groups Zn50 and Zn100 on day 42 *($P<0.05$)*. In group Zn100, significantly increased values of bone cross-section area (Ar) were observed on days 14 and 35 and moment of inertia (I) on days 14, 21 and 35 *($P<0.05$)*. Results suggested that the determination of the weight bearing of tibial bone evaluated by the determination of mechanical properties of bones is a good indicator of the locomotory system disorders and the Zn content in feed has a direct statistical significant effect on bone strength in broiler chickens.

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

INTRODUCTION

Orthopaedic-related leg problems contribute to a large percentage of profit loss in poultry industry (Rath et al., 2000). Fast growing hybrids might have different requirements of 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 amount 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

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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 shown 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. (2001) 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 of 30 to 60 mg/kg of feed (Jantošovič et al., 1998). Other authors reported that the optimum Zn range for broilers is 40 to 70 mg of Zn/kg feed (Underwood and Suttle, 1999) or up to 68 mg of Zn/kg feed (Wang et al., 2002). The data indicate that a relatively wide range of Zn content in food may affect the bone strength. A recommended amount of Zn for Ross broiler chickens in complete diet according to The Ross 308 Manual (2007) is 100 mg/kg of feed, despite this, many standard commercial poultry diets does not contain this required level.

The aim of this study was to asses the value of mechanical properties, determine the weight bearing property of tibial bone as indicator of the locomotory system disorders in broiler chickens and investigate the influence of different zinc levels in diet on direct mechanical characteristics of the chicken tibial bone.

MATERIALS AND METHODS

The study was carried out on 120 two days old chickens of Ross 308 hybrid without segregation according to sex. All birds were placed in 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/week until it reached 21°C which was maintained till the end of the experiment. All birds were divided into 2 groups; 60 chickens in each. All birds were fed diets with identical nutritional density and water was offered *ad libitum*. The feed given to broilers was in three phases: starter diet (1 to 14 days), grower diet (15 to 35 days) and finisher diet (36 to 42 days). The ingredients and nutrient composition of broiler starter, grower and finisher 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 diets *ad libitum* until day 42 of age. At weekly intervals (on days 7, 14, 21, 28, 35 and 42), the locomotory system of 10 broilers from each group was examined to find the skeletal disorders and then chickens were euthanized by cervical dislocation. The tibiotarsal bones of both legs were dissected from fresh carcasses and stripped off the soft tissues. They were stored in plastic bags at -20°C and warmed up to 20°C before the testing. Static tests of mechanical properties of bones were carried out on a universal tensile testing machine FP100/1 using a three point bending test at ambient temperature of 20°C (Baranová, 2008). The rate of loading was 2.5 mm.min^{-1}, the loading force ranging from 40 N to 1 kN 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 to 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 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.

RESULTS

In this study, the bending strength (Rm), limit of elasticity (Re) and fracture stress (Rl) changed significantly in Zn100. During the first 3 weeks of the experiment, the Re values in groups Zn50 and Zn100 persisted at approximately the same level (Table 3). From day 28 of the experiment in group Zn100, we observed a gradual increase in Re culmination on day 35, when the difference was statistically significant *(P<0.01)*. In group Zn100, the same trend was observed for Rm and Rl with the maximum values on day 35 *(P<0.05)*. Moreover, in the values of Rl, there was a significant difference between groups Zn50 and Zn100 on day 42 *(P<0.05)*. Consequently, from the 3rd week of the experiment, the chickens fed increased level of zinc showed a noticeable increase in the level of stress up to the point where the bone was deformed elastically (reversibly) and after unloading, it returned to its original shape and dimensions (=Re). Also, an increase was observed in the values of the highest bending stress on the surface of the flexed bone (=Rm) and in the values of the bending stress at the point of fracture (=Rl).

Table 1. Composition of the control broiler diet.

Ingredient	Starter	Grower	Finisher
Nitrogen substances, g/kg	200.0	180.0	170.0
Metabolizable energy, MJ/kg	12.0	2.0	12.0
Ash, g/kg	70.0	70.0	70.0
Fibrous material, g/kg	35.0	40.0	40.0
Methionine + cystine, g/kg	7.5	7.5	7.5
Methionine, g/kg	4.5	4.0	4.0
Lysine, g/kg	11.0	9.5	9.5
Ca, g/kg	8-14.0	8-14.0	8-14.0
P, g/kg	5.0	5.0	5.0
Na, g/kg	1.2-4.0	1.2-4.0	1.2-4.0
Mn, mg/kg	60.0	60.0	60.0
Fe, mg/kg	60.0	60.0	60.0
Cu, mg/kg	4.0	4.0	4.0
Zn, mg/kg	50.0	50.0	50.0
Vit. A, IU/kg	10 000	8000	8000
Vit. D3, IU/kg	2000	800	800
Vit. E, IU/kg	10.0	10.0	10.0
Vit. B2, mg/kg	4.0	3.0	3.0
Vit. B12, mcg/kg	20.0	20.0	20.0

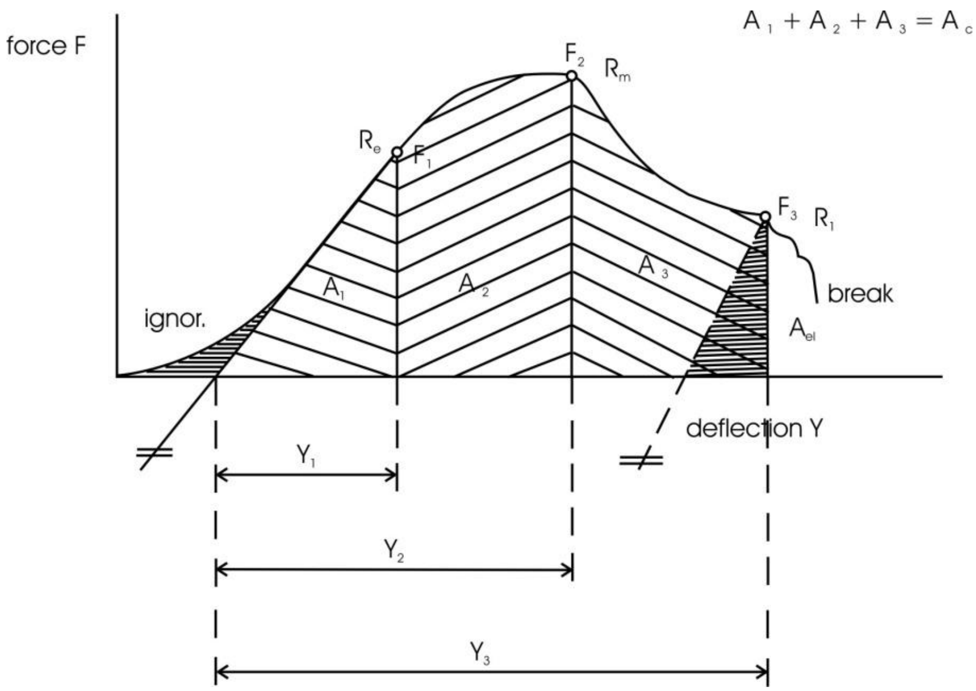

Figure 1. The bone force-deflection curve with indication of respective parameters: Re (limit of elasticity), Rm (bending strength), Rl (fracture stress), F1 (force at the limit of elasticity), F2 (maximum loading force), F3 (force in the moment of bone breaking), Y1 (bone deflection corresponding to 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).

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 . D³ – b . d³) . Л/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) . Л/4
Limit of elasticity (Re)	The stress up to which the bone is deformed elastically (reversibly)	Re (Mpa) = F1. s . D / (8 . I) F1 = force at the limit of elasticity s = distance between supports group 1 (7d old) =25 mm group 2 (14d old) =30 mm group 3 (21d old) =40 mm group 4 (28d old) =45 mm groups 5, 6 (35 and 42d old) = 60 mm
Modulus of elasticity (E)	Constant of proportionality between stress σ and deformation ε	E (Mpa) = F1 . s³/ (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 (W)	The work needed to deform the bone up to its failure	W (N/mm = 10^{-3} J/mm²) = (Ac – Ael)/Ar Ac = total work Ael = elastic energy

Our experiment also showed significant differences in the bone cross-section area at the point of fracture (Ar) which increased linearly in correlation with age. A significant increase in the values of Ar was detected in group Zn100 on days 14 and 35 *(P<0.05)*. The moment of inertia (I) increased gradually with the age. In this experiment, the level of I was higher in group Zn100 throughout the experiment, except for its first and last phases (days 7 and 42) when an opposite effect was observed. The differences between groups Zn50 and Zn100 were significant on days 14, 21 and 35 *(P<0.05)*. No significant differences between groups Zn50 and Zn100 were observed in the modulus of elasticity (E) and toughness (W) of bones. The level of E was slightly higher in the bones excised on days 7, 14 and 21 as compared to bones excised on days 28, 35 and 42. The values of W were almost the same in both groups with the exception of day 35.

The values of parameters Re, Rm and Rl in the bones excised on days 7, 14 and 21 reached lower levels as compared to the bones excised on days 28, 35 and 42. The value of I was age related and increased linearly with increasing age. The parameters of E and W were the least dependent on age. With parameters Re, Rm and Rl, the range of measured values was wider for bones excised on days 28, 35 and 42 and statistically significant differences were found more frequently for bones of broilers at the age of 7, 14 and 21.

In 6 broiler chickens from group Zn50, the examination of the locomotory system showed the varus deformities in the last phase of the experiment. In group Zn100, the bone deformities were not detected.

DISCUSSION

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 signify-cantly increased in 14 days old broilers fed diet with supplemental Zn (75 ppm). Scrimgeour et al. (2007) showed that the tibiotarsal bone as 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) of a chicken tibial bone

Table 3. Comparison of the limit of elasticity (Re), bending strength (Rm), fracture stress (Rl), toughness (W), 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, 14, 21, 28, 35 and 42 days.

		7 Days	14 Days	21 Days	28 Days	35 Days	42 Days
Zn50	Re (MPa)	61.00	61.68	58.60	84.14	94.27	86.46
	SD	10.04	10.17	11.12	10.00	18.71	29.28
	Rm (MPa)	73.82	79.29	77.60	93.08	100.2	93.33
	SD	9.89	11.89	13.27	11.95	20.56	29.54
	Rl (Mpa)	42.64	51.68	51.78	76.14	80.93	72.44
	SD	12.65	13.96	19.98	22.65	35.22	25.52
	W (N/mm)	13.35	12.07	10.81	11.27	8.95	13.77
	SD	2.72	4.16	5.11	6.06	7.26	8.35
	E (MPa)	3608	3637	3036	1791	2500	2454
	SD	1329.00	1307.00	891.00	689.30	578.50	1152.00
	Ar (mm²)	3.47	5.56	11.18	20.73	24.87	35.63
	SD	0.78	1.36	2.04	2.79	6.19	10.43
	I (mm^4)	1.45	3.86	16.03	49.03	88.47	173.40
	SD	0.40	1.57	4.11	11.42	35.49	94.17
	n[1]	20	20	20	20	20	20
Zn100	Re (MPa)	64.00	57.86	59.85	96.23	117.80**	98.65
	SD	7.98	8.12	15.80	36.89	19.66	27.20
	Rm (MPa)	76.68	75.70	84.09	104.00	126.70*	106.20
	SD	9.09	10.29	18.41	37.89	32.56	26.39
	Rl (MPa)	42.88	46.15	61.46	79.89	114.10*	92.58*
	SD	10.23	16.32	24.33	43.10	19.92	28.57
	W (N/mm)	13.14	12.65	9.26	14.34	6.27	11.43
	SD	4.76	3.16	4.42	10.70	3.84	7.47
	E (MPa)	3469	2745	2624	1856	2785	2263
	SD	630.90	705.90	826.10	689.30	752.80	704.70
	Ar (mm²)	3.18	6.74*	12.77	18.35	30.48*	30.04
	SD	0.52	1.76	3.06	4.55	4.47	8.05
	I (mm^4)	1.30	5.54*	20.46*	51.20	120.70*	142.70
	SD	0.34	2.56	7.57	17.47	30.37	66.21
	n[1]	20	20	20	20	20	20

[1]n = Number of values (n = 20); [2]SD = standard deviation.*****$P<0.05$; ****** $P<0.01$.

changed significantly in Zn100 which is agreement with the results of Shelton and Southern (2007). A noticeable increase in the level of Re, Rm and Rl from the 3rd week of the experiment in the chickens fed increased level of zinc can be due to culminant ossification of tibiotarsus in this particular phase of experiment. According to Adamec (2011), the epiphyseal ossification center in the proximal end of tibiotarsus is radiografically noticeable at the age of 35 days. The gradual increase in Re, Rm and Rl determined in our experiment agree with the results of Shelton and Southern (2007) and Scrimgeour et al. (2007) who reported an influence of Zn on the mechanical properties of bones. These authors also showed that in animals, lower zinc content in feed caused the reduction of bone integrity, bone density, 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 Wang et al. (2002), Shelton and Southern (2007), Nielsen et al. (1970) and Rossi et al. (1999, 2001).

Our experiment showed significant differences in the bone cross-section area at the point of fracture (Ar) and the moment of inertia (I) which increased linearly in correlation with age, and is agreement with the results of Baranová (2001). Baranová (2001) showed that I, as a very sensitive mechanical property of bone, is markedly influenced by the changes in the structure of bone. This author examined the mechanical properties of bones in different phases of age and described that the bones in older animals (at the age of 28, 35 and 42 days) tended to show wider range of measured values than small broiler bones (at the age 7, 14 and 21). The same results were shown in this experiment.

Skeletal problems associated with low zinc diet are reported by many authors (Jurajda, 2003; Herenda and Franco, 1996; Klimeš, 1970).

Conclusion

The present study confirmed the differences in bone bio-mechanical competence in broilers fed diet with different Zn levels. There were significant changes in mechanical properties of the bones, suggesting reduced weight bearing capabilities in broilers fed diet with zinc content of 50 mg/kg of feed as compared to chickens fed increased level of zinc (100 mg/kg of feed). In group with lower Zn content, the incidence of varus deformities was shown in 10% of the broilers. This experiment showed that the determination of the weight bearing of tibial bone evaluated by the determination of mechanical properties of bones is a good indicator of the locomotory system disorders and the Zn content in feed has a direct statistically significant effect on bone strength in broiler chickens The results suggest a beneficial effect of zinc supplementation on reduction of locomotory disorders in broiler chickens.

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