THE EFFECT OF DIETARY ZINC AND CALCIUM CONTENT ON THE FEMUR BONE STRENGTH OF BROILERS

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Abstract: In this experiment, two organic zinc (Zn) sources, zinc chelate of proteinate (Zn-proteinate) and zinc chelate of glycine (Zn-glycin) and three calcium (Ca) levels (4 or 9 or 14 g/kg) were evaluated for their effects on the strength of broiler femur bone. Total of 48 male broiler chicks Ross 308 were divided into six groups according to 2x3 factorial arrangement (2 zinc sources and 3 supplemented calcium levels). The basal diet contained 33 mg Zn/kg and 3.2 g/kg Ca and experimental premix was modified by adding 100 mg/kg Zn either as Zn-proteinate or Zn-glycin and by calcium levels to achieve total calcium content 4 or 9 or 14 g/kg in the diet. Calcium was supplied as $CaCO_3$. The feed mixture and water were offered ad libitum. The experiment started at 11 days of broiler age and chicks were fattened up to 35 days of age. At the end of the trial, birds were slaughtered and femur bone from right leg was dissected. The bone breaking strength as force in the moment of bone breaking was measured. The calcium level had significant effect ($P<0.05$) on the strength of broiler femur bone between group with Zn-glycin + 4 g/kg Ca and group Zn-glycin + 9 g/kg Ca, however zinc source had no significant effect.

Key Words: zinc, calcium, broiler, femur bone

INTRODUCTION

Breeding chickens for superior meat production is associated with higher body weight which loads chicken skeleton. Orthopaedic problems may cause reduced feed intake, weight gain, low productivity, mortality and economic loss. Weak bones can cause bone fractures during catching, transporting and processing of chicken meat (Rath et al. 2000).

In poultry management, nutrition is central to the maintenance of skeletal health. The extent of bone mineralization affects bone strength, and poor mineralization has been associated with increased risk of fractures. The strength of bones is affected by age, gender, nutrition, physical activity, infection and disease, hormones, toxins and antinutrients (Rath et al. 2000).

Calcium (Ca) together with phosphorus are primary inorganic nutrients in the bone, because they form 95% of the mineral matrices. Calcium deficiency can lead to skeletal deformation, rickets and bone fracture. Calcium deficiency does not seem to be a problem, but the interaction among calcium and other elements can impair Ca absorption (Rath et al. 2000). It is well established that zinc can permeate a variety of calcium channels and on the other side very high levels of calcium may inhibit zinc absorption (EFSA 2014). Zinc (Zn) is an essential trace mineral necessary for growth, bone development, feathering, reproduction, immune system, disease resistance, maintaining insulin levels, it is part of the list of zinc metaloenzymes. Deprivation of zinc is characterized by loss of appetite, growth depression, abnormalities of the skin or outgrowths (hair, wool, feathers, hoof, horn) and reproductive disorders (Suttle 2010). Deficiency of zinc in chicks can cause decreased growth, frizzled feathers, shortened and thickened legs or enlarged hocks (Nielsen 2012). Feed materials for poultry are usually either too low in zinc or show reduced availability of zinc to cover the animal requirements, so animal feeds are routinely supplemented with zinc (EFSA 2014). Zinc is added to broiler diets in inorganic sources (usually zinc oxide, zinc sulphate, zinc chloride) or in organic forms complexed to amino acids, proteins, or carbohydrates.

The National Research Council (1994) estimated the dietary requirement for broilers as 40 mg Zn/kg and 9 g Ca/kg. Recommended values are usually higher than NRC requirements for ensuring adequate performance. According to Ross Broiler Management Handbook (2014) recommended added zinc content is 110 mg/kg of the diet. Recommended calcium content is 8.7 g/kg complete diet from 11 to 24 days of age and 7.9 g/kg from 25 days of age.

The aim of this study was to investigate the influence of different zinc sources and calcium levels in the diet on strength of broiler femur bone.

MATERIAL AND METHODS

The experiment was conducted with 48 male chicks of hybrid Ross 308. Birds were marked by wing tags and housed in the balance cages in a room that had a temperature set according to Ross Broiler Management Handbook (2014). The chicks were divided into 6 groups and they had free access to feed and water throughout feeding trial. The lighting system was set on 18 hours light and 6 hours dark. Temperature and relative humidity was recorded every day. The experiment started at 11 days of broiler age and chicks were fattened up to 35 days of age.

Birds were fed by the diet (composition of the diet is shown in Table 1) formulated to meet or exceed NRC (1994) nutritional requirements except zinc and calcium. It was used a modified vitamin-mineral premix with minimum amount of zinc and calcium. The basic diet contained 33 mg/kg Zn and 3.2 g/kg Ca, originated from feedstuffs and the zinc-calcium-low premix. The experimental premix was modified by adding 100 mg Zn/kg either as Zn-proteinate or Zn-glycin and by calcium levels to achieve total calcium content 4, 9 or 14 g/kg in the diet. This was achieved by adding different amounts of $CaCO_3$. Dietary treatments are shown in Table 2.

Table 1 Composition of the diet

Ingredient	g/kg
Maize	260
Wheat	342
Soybean meal	305
Rapeseed oil	40
Vitamin-mineral premix [1]	20
Experimental premix[2]	30
Chromium oxide	3

[1]Supplied per kilogram of premix: lysine 101.65 g, methionine 135.63 g, threonine 51.22 g, calcium 200 g, phosphorus 98.19 g, natrium 62.89 g, sulphur 0.39 g, chlorine 119.69 g, copper 752.5 mg, iron 3768.6 mg, zinc 44.73 mg, manganese 6046.07 mg, cobalt 11 mg, iodine 47.95 mg, selenium 8.96 mg, vitamin A 680000 IU, vitamin D 250000 IU, vitamin E 2250 mg, K_3 74.8 mg, B_1 206.44 mg, B_2 344 mg, B_6 300.44 mg, B_{12} 1999.2 mg, biotin 11 mg, niacinamid 1793.4 mg, calcium pantothenate 676.2 mg, folic acid 82.8 mg, cholinechlorid 9000 mg
[2] Content different sources of zinc and levels of calcium according to the dietary treatments

Table 2 Added zinc and calcium content in the dietary treatments

Group	Zinc source	Zn (mg/kg)	Ca (g/kg)
P4	*Zn-proteinate*	100	4
P9	*Zn-proteinate*	100	9
P14	*Zn-proteinate*	100	14
G4	*Zn-glycin*	100	4
G9	*Zn-glycin*	100	9
G14	*Zn-glycin*	100	14

At the end of the feeding trial (35 days of broiler age), birds from each group were slaughtered and femur bone from right leg was dissected from fresh carcass and stripped of soft tissues. They were stored individually in plastic bags in a freezer (-20 °C) for 3 weeks and then they were thawed at room temperature the day before tests of strength. The tests of strength of bones were carried out on a universal testing machine TIRATEST 27025 using a three point bending test. The bones strength were measured as the force in the moment of bone breaking. Breaking force was determined automatically by the device. The loading rate was 100 mm/min up to moment of bone breaking.

Data has been processed by Statistica version 12.0 (CZ). We used two-way analysis (ANOVA). Sheffe´s test was applied to defined statistical differences and differences were considered significant at P < 0.05.

RESULTS AND DISCUSSION

In this experiment, it was added 100 mg Zn/kg diet from two different sources: Zn-proteinate and Zn-glycin. The strength of femur bone was not significantly affected by these zinc sources. The breaking force was significantly affected by calcium level (P<0.05). Significant differences (P<0.05) were recorded between groups G9 (307.7 N) and G4 (175.2 N) with diet contain 9 g Ca/kg and 4 g Ca/kg diet, both supplemented by Zn-glycine. The effects of supplemented zinc sources and calcium levels in this study on the strength of broilers femur bones are presented in Table 3.

Table 3 The effects of dietary zinc and calcium on the strength of broilers femur bones

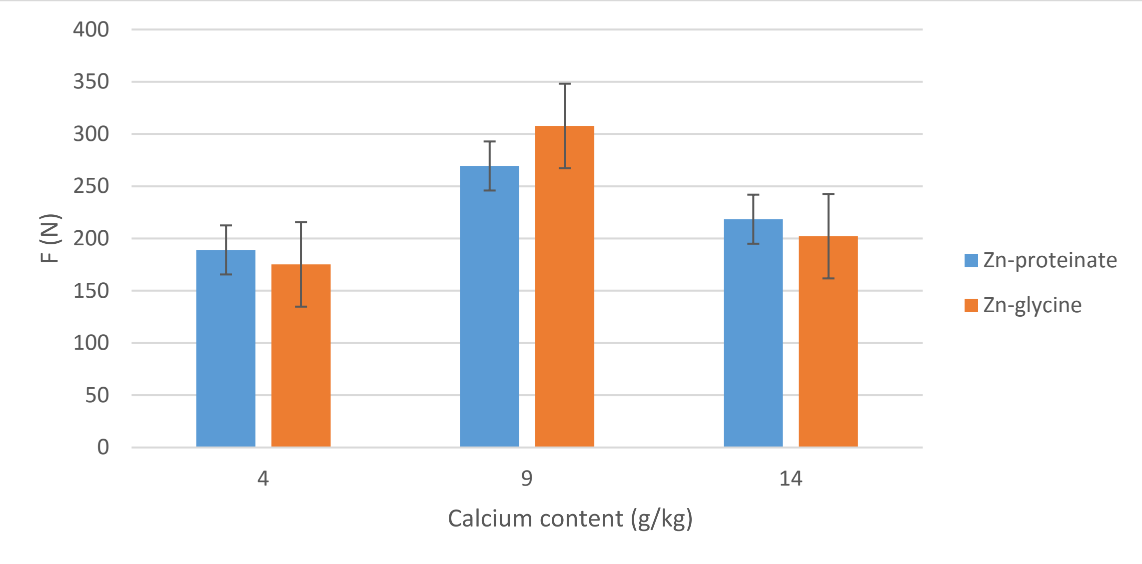
Group	Zinc source	Calcium level (g/kg)	Breaking force (N) ± SD		
P14	Zn-proteinate	14	218.5	±	61.2
P9	Zn-proteinate	9	269.4	±	12.0
P4	Zn-proteinate	4	189.0	±	38.7
G14	Zn-glycine	14	202.2	±	52.2
G9	Zn-glycine	9	307.7 [a]	±	40.2
G4	Zn-glycine	4	175.2 [b]	±	19.0
Zn source			NS		
Ca level			*		
Zn source x Ca level			NS		

*NS: not significant; * P<0.05; Different letters [a,b] in the columns indicate significant differences at a level of P<0.05*

The bone strength is affected by the extent of bone mineralization. Many studies were realized to determine the influence of dietary zinc on concentration of zinc in tibia bones. Scrimgeour et al. (2007) claimed that the tibiotarsal bone compared to the femoral bone is more sensitive to different Zn levels. Underwood and Suttle (1999) found a decrease in Zn concentration in bones caused by lower zinc content in the feed and Mohanna and Nys (1999) recorded that tibia zinc content increases with dietary zinc level. Štofaníková et al. (2011) confirm the differences in bone biomechanical competence in broilers fed diet with different zinc levels. The significant reduction in tibial strength was observed in broilers fed 50 mg Zn/kg in comparison with 100 mg Zn/kg. Rama Rao et al. (2006) recorded tibia breaking strength significantly higher by increasing the dietary Ca level from 6 to 7 g Ca/kg diet. Lander et al. 2014 dealt with the effect of freezing (at −20 °C for 21 days) on the microstructure of bone, and they found microcracks and cracks on the surface of the frozen bone samples, but no significant differences were observed. Similarly Tersigni (2007) also reported no significant microstructural changes on frozen bone samples and control samples.

In our study, maximum breaking force occurred at a calcium content of 9 g/kg diet and then showed declining trend with increase in dietary Ca level (14 g/kg). The lowest values was found in groups G4 and P4 with calcium content of 4 g/kg feed mixture independently of zinc source (Figure 1).

Figure 1 The effects of dietary zinc and calcium on the femur bone strength of broilers



F = force in the moment of bone breaking

The strength of femur bone was not improved when calcium was increased from 9 to 14 g/kg diet. There is some other contraindicative factors except zinc which can interfere with Ca absorption, such as high levels of phytate and cellulose fibers in the diet. (Rath et al. 2000).

CONCLUSION

In this experiment, different zinc sources and calcium levels were evaluated for their effects on the breaking strength of femur bone of broiler at the age of 35 days. This parameter increased independently of zinc source from calcium content of 4 g/kg and 14 g Ca/kg and the best results were achieved at level of 9 g Ca/kg. In case of zinc sources, zinc bounded to proteinate suggested better results with 4 g Ca and 14 g/kg, however the best strength of femur bone was noticed in group with zinc bounded to glycin and 9 g Ca/kg. Zinc source had no significant effect on breaking force. The increase of calcium content from 4 to 9 g/kg in Zn-glycine groups significantly ($P<0.05$) increased force required for bone breaking.

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