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Original Article

Effect of Dietary Growth Promoter Supplementations on the Serum Biochemistry and Morphometric Characteristics of Tibia Bone in Broiler Chicken

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ABSTRACT

Bone mineralization is considered an essential source of minerals for metabolic needs that provide strength and hardness to the bone tissues and in birds its development can be enhanced by the use of dietary supplements. The present study was executed to evaluate the influence of prebiotic, probiotic and antibiotic on the morphometric aspects of tibia bone in broilers. One-hundred and sixty-one-day-old broiler chick were divided randomly into four groups (n=40) with 4 replicates (n=10 in each replicate). Group-I fed with only corn based basal diet and served as controlled group. Group-II with antibiotic supplement (Zinc bacitracin 0.04%), Group-III with probiotic supplement (lactobacillus 0.1%) & Group-IV with prebiotic supplement (mannan oligosaccharide 0.1%). On the 35th day, two birds were selected randomly from each replicate and slaughtered to record the morphometric parameters of both right and left tibia bones from each bird. The findings showed that the length, weight, thickness of lateral and medial wall, tibiotarsal index of bone and bone ash percentage of supplemented groups have had significant (p<0.05) difference when compared to that of the controlled group. The weight/length index in birds supplemented with prebiotic and probiotic was significantly higher (p<0.05) than in the antibiotic supplemented and control group. Medullary canal diameter of all the supplemented groups was significantly lower (p<0.05) than the control group. No significant (p>0.05) difference of robusticity index and diaphysis diameter was observed among various groups. A significant (p<0.05) low level of Alkaline Phosphatase while higher level of Calcium and Phosphorus was recorded in the supplemented groups compared to that of the control group. In conclusion, the use of prebiotic and probiotic as growth promoter can exhibit some underline mechanisms in the form of enhancement of bone quality, density and characteristics in broiler chicks as compared to common antibiotics.

INTRODUCTION

Metabolically, bone is a vital structure and its growth is affected by the absolute growth rate, nutrition, aging and genetic factors (Hauge *et al.*, 2001). The skeletal system provides the structural posture to the body that protects the internal body organs and facilitates in locomotion, it also serves as a source of reserved minerals (Kocabagli, 2001). Strength and stiffness of bone is due to phosphorus, manganese and calcium (Onyango *et al.*, 2003) while the leg bone distortion negatively influenced the growth efficacy of birds (Sahraei, 2014; Talaty *et al.*, 2010). Increased mineral deposition has a beneficial correlation with mechanical strength and density of bone (Schreiweis *et al.*, 2003; Mirakzehi *et al.*, 2018). Bone defects in the mode of mortality may lead to economic losses (Rath *et al.*, 2000).

The utilization of antibiotics in feed as a growth booster has favorable impact on bird growth efficiency by supplying supplementary nutrients (Mile *et al.*, 2006; Oliveira *et al.*, 2009). Complications like antibiotic residual side effects in animal products and antibiotic resistance play a main role in shifting from antibiotics to some nontoxic substitutes such as prebiotics, probiotics, organic acids and phytobiotics (Patterson & Burkholder, 2003). Probiotics are described as culture of live substance and micro-organisms that positively affect the host by maintaining and manipulating the gut micro flora (Alloui *et al.*, 2013). Probiotic supplementation leads to fortifying the bone density and bone mineralization by improving the mineral's digestibility (Mutus *et al.*, 2006). Moreover, prebiotics are non-absorbable components of diet that can boost the activity and growth of helpful essential microbes and positively influence the animal´s health by availability of nutritional components, increasing digestibility and lower the harmful micro flora in the GIT (Alloui *et al.*, 2013). Prebiotics enhance the bone mineralization and detention of calcium in broilers (Kim *et al.*, 2006). If we are able to draw the comparative efficacy of probiotics, prebiotics and antibiotics for morphometric characteristics of tibial bone, the practical benefits would be enormous for better commercialization of broiler chicks. The present research has been planned to assess the relative efficacy of antibiotic, prebiotic and probiotic on bone morphometric features and serum biochemistry in broiler chicken.

MATERIALS AND METHODS

Experimental Birds and Housing

One-hundred-and-sixty-day-old broiler chicks were obtained from a local retail shop. The chicks were weighed and raised under environmentally controlled conditions. The birds were distributed randomly into four groups (n=40) with 04 replicates (in each replicate n=10). The control group was fed only the basal diet (Table 1) named as Group-I. Group-II was fedwith Antibiotic supplemented feed (Bacitracin as Zinc bacitracin 0.04%), Group-III was fed probiotic supplemented feed (Alterion aslactobacillus 0.1%) and Group-IV with prebiotic treated feed (Green culture as mannan oligosaccharide 0.1%). The relative humidity and temperature on day 01 were maintained at 70 ± 5% and 35 ± 1.5 °C, respectively. Relative humidity was lowered 65 ± 5% up to the completion of the research study; with temperature decrease of 2-3 °C per week until it reached 26.7 °C on day 21.

Table 1 – Ingredient composition of the basal diet.

Ingredients	Ingredient percentage (%)
Corn	28.08
Rice tips	24.74
Canola meal	4.01
Rape seed meal	3.34
Soybean meal	28.08
Corn gluten meal 60%	2.01
Poultry product meal	3.34
Chips	0.67
L-Lysine sulphate 70%	0.67
MHA	0.29
L-Therionine	0.12
Cooking oil	3
Salt	0.31
Bone ash	1.2
Pre-mix	0.14

Sample collection

Eight birds from each group (2 birds per replicate) were selected randomly and slaughtered by incising the carotid artery. Blood samples were collected in corvac tubes to calculate the level of Calcium, Phosphorus and alkaline phosphatase in blood serum. The left and right tibia bones as drumstick with muscular tissue of each bird were separated, labelled and soaked in boiling water (100 °C) for 10 minutes. Specimens were made cold at room temperature after withdrawing from the boiling water. Meat from each drumstick was removed manually. Bones were dried for 24 hours at room temperature and weighed by digital weighing balance (Mutus *et al.*, 2006). Diaphysis diameter and length of each bone were determined with digital caliper. After breaking the bones at midpoint, thickness of lateral wall, thickness of medial wall and medullary cavity diameter were determined. The weight/ length index of bone was calculated by dividing the weight of bone by its length. Furthermore, bone ash percentage was calculated related to dry bone weight (Rath *et al.*, 2000).

Robusticity index and tibiotarsal index were calculated by the given equations,

Robusticity index = bone length/cube root of bone weight (Reisenfeld, 1972).

Tibiotarsal index = [diaphysis diamater – medullary canal diameter/diaphysis diameter] x 100 (Barnet & Nordin 1960).

Serum was separated by centrifugation for 5 minutes at 3000 rpm. Calcium, Phosphorus and Alkaline phosphatase level in serum were determined by chemistry analyzer.

Statistical Analysis

The data was analyzed by computer software SPSS for windows (Statistical package for social sciences, Inc., Chicago, IL, USA, Version 20) using one-way analysis of variance and the results were declared as mean ± S.E. Tukey's Range Test was used to compare the group differences (Steel & Torrie, 1980). Group variations were assumed significant at $p<0.05$.

RESULTS

The findings of groups supplemented with different feed additives on tibia bone's morphometric features in broiler chicks are presented in table 2. The findings showed that length, weight, tibiotarsal index, thickness of lateral wall and thickness of medial wall of birds fed with probiotic (lactobacillus 0.1%), mannan oligosaccharide and zinc bacitracin showed significantly higher values ($p<0.05$) than the controlled group. Furthermore, the bone weight/bone length index of broiler chicks fed with probiotic and prebiotic presented significantly ($p<0.05$) higher values than the zinc bacitracin supplemented and control group. The diameter of the medullary cavity of all supplemented groups was lower than the control group.

Robusticity index and diaphysis diameter showed no significant ($p>0.05$) difference between all supplemented groups and the control group. Bone weight and weight/length index of broiler chicken supplemented with probiotic (lactobacillus 0.1%) were significantly increased ($p<0.05$) as compared to antibiotic supplemented group. Bone ash percentage of broiler chicks supplemented with mannan oligosaccharide (0.1%), lactobacillus-based probiotic, and zinc bacitracin were significantly higher ($p<0.05$) than the bone ash percentage of the control group.

Table 2 – Effects of dietary supplementations on morphometric characteristics of tibia bone.

Parameters	Group-I Control	Group-II ZnB	Group-III LBP	Group-IV MOS
Weight (grams)	3.08^{c}± 0.09	3.52^{b} ± 0.11	4.07^{a} ± 0.01	3.85^{ab} ± 0.14
Length (mm)	73.75^{b} ± 1.26	78.39^{a} ± 1.20	79.13^{a} ± 0.92	79.49^{a} ± 1.18
Diaphysis Diameter (mm)	7.41^{a} ± 0.31	7.53^{a} ± 0.33	7.62^{a} ± 0.28	7.52^{a} ± 0.26
Medullary canal Diameter (mm)	5.03^{a} ± 0.07	4.10^{b} ± 0.10	4.28^{b} ± 0.67	4.44^{b} ± 0.12
Medial wall thickness (mm)	0.79^{b} ± 0.03	1.23^{a} ± 0.07	1.32^{a} ± 0.08	1.19^{a} ± 0.05
Lateral wall thickness (mm)	1.40^{b} ± 0.07	2.27^{a} ± 0.12	2.17^{a} ± 0.09	2.04^{a} ± 0.08
Bone ash %	42.44^{b} ± 0.32	44.69^{a} ± 0.28	44.94^{a} ± 0.49	44.94^{a} ± 0.27
Weight/length index(mg/mm)	41.78^{c} ± 1.48	44.91^{b} ± 1.41	51.64^{a} ± 0.10	48.50^{ab} ± 1.82
Tibiotarsal index	32.49^{b} ± 1.94	45.34^{a} ± 1.78	44.24^{a} ± 1.25	42.14^{a} ± 1.02
Ribusticity index	5.08^{a} ± 0.10	5.16^{a} ± 0.09	4.95^{a} ± 0.04	5.08^{a} ± 0.09

ZnB=0.04% Zinc bacitracin, LBP=0.1% Lactobacillus based probiotic, MOS=0.1% Mannan oligosaccharide.

Results of different supplemented groups on serum biochemistry are presented in table 3. The results revealed that alkaline phosphatase level of all groups supplemented with growth promoters was significantly lower ($p<0.05$) than the controlled group. Calcium and Phosphorus in blood serum of all dietary groups were significantly increased ($p<0.05$) as compared to the controlled group.

Table 3 – Effects of dietary supplemented growth promoters on blood serum biochemistry.

Parameters	Group-I Control	Group-II ZnB	Group-III LBP	Group-IV MOS
Calcium (mg/dl)	5.97^{c}± 0.03	6.21^{b} ± 0.03	6.29^{ab} ± 0.03	6.31^{a} ± 0.03
Phosphorus (mg/dl)	2.95^{c}± 0.03	3.13^{b} ± 0.03	3.31^{a} ± 0.03	3.37^{a} ± 0.03
Alkaline Phosphatase (IU/L)	51.81^{a} ± 1.01	47.84^{b} ± 1.21	47.19^{b} ± 0.57	47.13^{b} ± 0.83

Mannan oligosaccharide

DISCUSSION

Tibia bone length, weight, thickness of the external wall, bone ash percentage and thickness of the internal wall were significantly boosted by the dietary antibiotic supplemented group when compared to the controlled group (Ziaie *et al.*, 2011) which help the results of our research trial. In contradiction, Sabah (2011) described that the density of the medial wall, weight, the length of bone and density of the lateral wall were not improved by the probiotic or antibiotic supplementations. Furthermore, probiotic supplementation did not affect the bone weight/length index but the compactness of internal & external wall and the bone ash percentage were significantly increased (Mutus *et al.*, 2006).

Availability and digestibility of minerals is enhanced by feed supplemented with antibiotics which helps to improve the mineral level of blood that leads to

an increase in resistance to fracture in bone and bone ash percentage (Dannie & Southern 2005). The amount of bone ash percentage is truly the indicator of mechanical bone strength, Bonser & Casinos (2003). Hosseini *et al.* (2016) reported that the plant extracts are involved in bone calcification through better retention of dietary calcium. Furthermore, it was confirmed in the study of Mirakzehi *et al.* (2018) which suggested that vitamin D3 in combination with plant extract showed significant amount of tibia mineralization and calcification. There is a beneficial correlation among bone ash and bone fracture (Kim *et al.*, 2006). The effects of mannan oligosaccharide and enzymes on morphometric characteristics of bone in broiler chicken were reported by Oliveria *et al.* (2009). They pointed out that the use of enzymes as growth promoter decreased the bone weight. According to earlier research trials, it could be decided that prebiotic, probiotic or antibiotic supplementation may positively affect the digestibility and availability of minerals which results in a significant enhancement in bone ash percentage.

Monteagudo *et al.* (1997) and Libouban *et al.* (2001) described that bone density has a direct correlation with bone weight/length index if index is large then bone is denser. Birds fed with antibiotics supplemented feed showed higher values of weight/length index than the controlled group (Ziaie *et al.*, 2011). Improvement in bone weight/length index of groups supplemented with growth promoters is due to enhance in bone weight.

Mutus *et al.* (2006) reported that bone mineralization has a positive interaction with tibiotarsal index. Broilers fed antibiotic replacements and virginiamycin supplemented feed showed high tibiotarsal index as compared to the control group (Ziaie *et al.*, 2011). Broiler birds fed diets supplemented with probiotic (lactobacillus 0.1%), antibiotic (Zinc bacitracin 0.4%) and prebiotic (mannan oligosaccharide 0.1%) showed decreasing values of medullary canal diameter as compare to the control group. Ziaie *et al.* (2011) confirmed these results and also revealed that bone medullary cavity diameter of the controlled group was significantly increased ($p<0.05$) as compare to the birds fed supplemented diets. Robusticity index also showed the bone fracture strength. Bone density is low and bone is fragile, if robusticity index is high (Reisenfeld, 1972).

The findings of blood serum alkaline phosphatase obtained in the current research trial were similar to the results of Hatab *et al.* (2016) and Aluwong *et al.* (2012) who presented a significant decrease in the level of blood serum alkaline phosphatase of broilers fed with probiotic growth promotors than the control group. Furthermore, the significant increases in the level of phosphorus and calcium in all the supplemented groups are similar to a study conducted by Strompfova *et al.* (2006). In addition, Scholz *et al.* (2007) reported that probiotic based growth promotors can significantly increase the absorption of calcium from the intestinal mucosa. While Hashemzadeh *et al.* (2013) reported that blood serum phosphorus and calcium levels are not significantly affected by the use of probiotics in broiler chickens.

CONCLUSION

The findings of the research trial indicated that prebiotic, probiotic and antibiotic supplemented feeds can enhance the morphometric aspects of bone, density, bone strength and blood serum parameters in broiler chicken. The use of prebiotic and probiotic as growth promoters are suspected to be the good alternative for improvement of bone characteristics, maintenance of healthy skeleton, bone quality and bone density in broiler chicken.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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