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Effect of Antibiotic and its Alternatives on Morphometric Characteristics, Mineral Content and Bone Strength of Tibia in Ross Broiler Chickens

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Abstract: An experiment was conducted using 360 one-day old male Ross (308) broiler chickens to study the effect of dietary supplementation of antibiotic alternatives growth promoters on morphometric characteristics, mineral content and bone strength of tibia. Chickens were fed in a completely randomized design with 4 replicate. Experimental treatments were: 1) control, 2) control + 15 ppm of Virginiamycin antibiotic, 3) control +150 m/kg of probiotic, 4) control + 100 mg/kg of Immnuwall prebiotic, 5) control + 450 mg/kg of commercial herbal blend and 6) control + 400 mg/kg of organic acid. Results showed that diaphysis diameter was not affected by the dietary supplementation of growth promoter components, whereas weight, length, tibiotarsi index, thickness of the medial and lateral wall of the tibia, tibiotarsal index, mechanical parameters, ash percentage, Ca and P contents were significantly improved by the supplementation antibiotic and their alternatives ($P<0.05$). Control diet had greater medullary canal diameter of the tibia than other groups. These data indicated that supplementation broiler diet with antibiotic and its alternative such as prebiotic, probiotic, commercial herbal blend and organic acid could improve bone characteristic.

Key words: Antibiotic Alternatives · Bone Index · Chicken

INTODUCTION

Bone status is commonly used as an indicator of mineral adequacy in poultry diets. The major mineral that form the inorganic matrix of bone are Ca and P [1]. Normal bone development in birds is also influenced by nutritional factors, genetic, gender, aging and the absolute growth rate. The skeleton not more only provides structural support for the bird but also is an important mineral source for metabolic needs [2]. Reichmann and Connor [3] showed that the extent of bone mineralization affects on bone strength and poor mineralization has been associated with increased risk of fractures. Weak bones result in breaking during processing and lower meat grade. Also, weak legs often result in reduced feed intake thus affecting weight gain and feed conversion ratio [4]. Both invasive and noninvasive methods have been used to evaluate bone mineralization in poultry. Invasive methods include bone ash, bone breaking strength, bone weight and bone volume [5].

The prophylactic use of antibiotic (as growth promoters) in animal feeds such as Virginiamycin has made intensive farming possible, improved feed conversion ratio [6] and digestibility of Ca, P and Mg of diet [7]. In the presence of low levels of antibiotic, resistant cells survive and grow, producing an antibiotic-resistant population. Consequently, the use of antibiotic for broilers has been limited in European Union to only 4 antibiotics that are not associated with human treatment [8]. Therefore, searches for alternative products that can aid in promotion of growth, improved feed utilization and maintenance of gut health are taking place. The primary alternatives to enhance gut function and growth performance to date include acidification of feed, feeding probiotic organisms, prebiotic compounds [9],

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herbal medicine and blends essential oil [10]. Probiotics defined as live microbial feed supplement that beneficially affects that host animal by improving its intestinal microbial balance [11] have been administered to counteract such stresses which subsequently improve body weight gain, feed conversion ratio and mortality rate in broiler chickens [12-14]. The result of researches on periodic[15], herbal medicines and their essential oil [10] and organic acid [16] showed that dietary administration of antibiotic alternative growth promoters improved body weight gain, feed efficiency ratio and ileal digestibility of nutrients in chicks. The objective of the present study was to investigate the effects of antibiotic growth promoters (AGP) and its alternatives on tibial bone characteristics of male Ross broilers.

MATERIALS AND METHODS

A total of three hundred and sixty one-day old male broiler chickens (ROSS 308) purchased from a local commercial hatchery, were weighed and randomly assigned into 6 groups of 60 birds each, each group being further separated into four replicates of 15 birds each. The feeding program consisted of starter diet until 21d of age and a finisher diet until 42 d of age. The composition of the experimental basal diet is shown in Table 1. Diets were formulated to met or exceeded requirements by the National Research Council [17] for broilers of this age.

The data on tibia bone parameters were analyzed as a completely randomized design using the GLM (General Linear Model) procedure of the SAS [20]. Differences between means were compared using the by Duncan Multiple Range Test (DMRT). Probabilities less than 0.05 were taken to indicate statistical significance.

Experimental diets were analyzed for ash, CP, Ca and P [19]. Teatments were: 1) control diet based on corn and soybean meal; 2) Control diet+ 15 ppm antibiotic (Virginiamycin); 3) Control diet+ 150 mg/kg diet probiotic(Protexin); 4) Control diet + 100 mg/kg diet mananoligosaccharide prebiotic (Immnuwall); 5) Control diet+ 450 mg/kg diet blend of medicinal plants (Digestrom); and 6) Control diet+ 400 mg/kg diet organic acid (commercial mixture of propionic acid, NH_3 and sodium bantonite). Protexin included *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus, Lactobacillus bulgaricus, Bifidobacterium bifidum, Enterococcus faecium, Candida pintolepessi, Streptococcus thermophilus, Aspergillus oryzea* with a minimum of 2×10^9cfu/g of product. Fresh feed and water were provided daily and were available *ad libitum*. The temperature of animal

Table 1: Ingredient (g/kg) and chemical composition of experimental diets

Ingredient	Starter 1-21 d	Finisher 22-42 d
Yellow corn	563	659
Soybean meal	310	263
Fish meal	48	18
Oil vegetable	35	21
Wheat bran	10	10
Dicalcium phosphate[1]	16.25	9
Oyster shell	8	9.5
DL-Methionine	2.225	2
Lysine	1.5	1.75
Vitamin premix[2]	2.5	2.5
Mineral premix[3]	2.5	2.5
Salt	1	1
Chemical composition		
ME kcal/kg	3000	3000
Crude protein %(analyzed)	22	19
Crude fat %	6.75	4.95
Methionine %	0.48	0.44
Lysine %	1.38	1.13
Met+Cys %	0.92	0.85
Tryptophan %	0.28	0.19
Calcium % (analyzed)	1.03	0.85
Phosphorus %(analyzed)	0.44	0.40

1. Contains 24% Ca and 17.5% available P.

2.Supplies per kg diet: Vitamin A, 16,500 IU; vitamin D_3, 3200ICU; vitamin E, 12 mg; vitamin K, 2 mg; vitamin B_1, 1.2 mg; vitamin B_2 10 mg; vitamin B_6, 2.4 mg; vitamin B_{12}, 12µg; niacin, 18 mg; pantothenic acid, 12 mg; 2.Mn, 90 mg; Zn, 72 mg; Fe, 60 mg; Cu, 10 mg; I, 1.2 mg; Se, 0.1mg facility on the first day of life was maintained at 31-33°C and lowered by 2-3 °C every week until 22-23°C in the final week. Continuous lighting was provided throughout the experiment. At age 42 d, five birds from each experimental unit were randomly selected and their left and right tibia were kept at-20 centigrade degree. Bone parameters (ash %, Ca and P %, length, weigh and morphometric and mechanical measurements) were determined for the samples. Preparation of the bones for the measurement of the parameters was undertaken based on Mutus, *et al.* [19]. The percentages of Ca and P of tibia were measured based upon AOAC [18]. Medial and lateral thicknesses of the samples were measured using dial caliper. Diameter of medullary canal computed from the difference between internal and external diameter of diaphysis. Indexes of tibiotarsal length and tibiotarsi length were calculated based on the following formulas:

Tibiotarsal length = [(Diaphysis diameter-medullary canal diameter)/(diaphysis diameter)]*100 (Barnet and Nordin, 1960);
Tibiotarsi length = Weight of bone / bone length

The medulus of bone elasticity and yield stress, were determined by the method described by Kocabagli, *et al.* [2].

RESULTS

Morphometric Parameters of Tibia Bone: Results for The effect of diet on morphometric parameters of tibia bone at 6 wk of age are shown in (Table 2). Supplementation of diets with growth promoters increased significantly (P<0.05) weight and length of tibia bone. No difference was found between diets containing antibiotic alternatives and virginiamycin. In this study, the highest and lowest tibiotarsi indices were found for probiotic and control diets respectively. Supplementation of diet with antibiotic and its replacements caused a significant (P<0.05) increase of the tibiotarsi index. Similar to tibiotarsi index, diets with virginiamycin or its replacements showed a significant (P<0.05) increase of the tibiotarsal index as compared to the control diet. Virginiamycin and control diets caused the greatest (8.64 mm) and lowest (8.05 mm) diaphysis diameter respectively and no significant differences were found among treatments applied in the experiments. For external and internal wall diameter, supplemented diets showed significant (P<0.05) increase compared to control diet. However, diet with virginiamycin had no significant difference with other treatments. Modular diameter was significantly (P<0.05) lower for growth promoter components compared to control diet.

Ash, Ca and P of Tibia Bone: The effects of feeding antibiotic alternatives on ash%, Ca% and P% of tibia bone are shown in (Figure 1). The broilers fed diets supplemented by growth promoter components had greater (P<0.05) percentage of tibia bone ash, Ca and P as compared to control diet.

Modulus of Elasticity and Tibia Yield Stress: Results for modulus of elasticity and tibia yield stress are shown in figure 2 and 3 respectively. Tibia bone modular elasticity and yield stress of chicks fed with growth promoter components was significantly (P<0.05) higher than the control diet.

Table 2:The effects of diets on morphometric parameters of tibia bone at 6 wk of age

	Treatment[1]						
Parameter	T1	T2	T3	T4	T5	T6	SEM[2]
Weight, g	4.42[b]	4.10[a]	5.23[a]	5.14[a]	5.09[a]	5.10[a]	0.15
Length, cm	88.19[b]	91.53[a]	91.72[a]	91.02[a]	91.86[a]	91.28[a]	0.98
Tibiotarsi wt/ length index, mg/cm	50.11[b]	55.76[a]	56.30[a]	56.46[a]	55.47[a]	55.97[a]	1.49
Diaphysis diameter, mm	8.05	8.64	8.54	8.46	8.46	8.31	0.24
Thickness of the medial wall, mm	1.41[b]	1.72[a]	1.73[a]	1.71[a]	1.73[a]	1.68[a]	0.084
Thickness of lateral wall, mm	2.53[b]	2.88[a]	3.10[a]	2.91[a]	2.96[a]	2.89[a]	0.084
Medullary canal diameter, mm	5.33[a]	4.58[b]	4.57[b]	4.54[b]	4.56[b]	4.62[b]	0.072
Tibiotarsal index	33.38[b]	46.72[a]	46.22[a]	46.07[a]	45.92[a]	44.12[a]	1.78

[a, b] Means with different superscripts within the same row differ significantly (*P*<0.05).

1. T1= control, T2= control + 15 ppm of Virginiamycin, T3= control + 100 mg probiotic Protexin/kg diet, T4= control + 100 mg prebiotic Immnuwall/kg diet, T5= control + 450 mg commercial herbal blend (Digestrom)/kg diet, T6= control + 400 mg of organic acid (Formycine) /kg diet.

2. SEM = Standard error of mean.

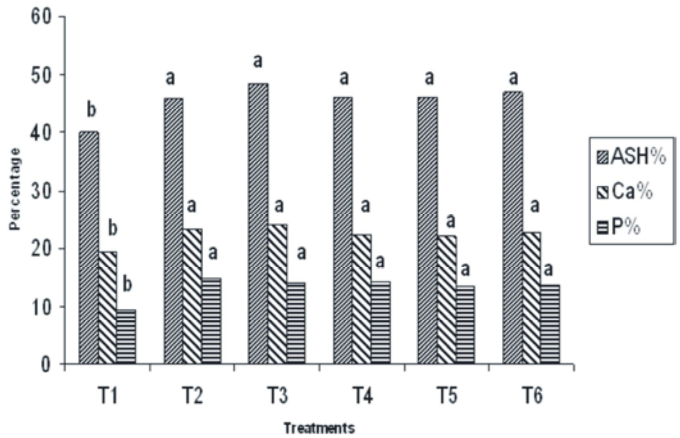

Fig. 1: Effects of feeding antibiotic alternatives on ash%, Ca% and P% of tibia bone

T1= control, T2= control + 15 ppm of Virginiamycin, T3= control + 100 mg probiotic Protexin/kg diet, T4= control + 100 mg prebiotic Immnuwall/kg diet, T5= control + 450 mg commercial herbal blend (Digestrom)/kg diet, T6= control + 400 mg of organic acid (Formycine) /kg diet.

For each parameter, values not having a common letter are significantly different ($P < 0.05$).

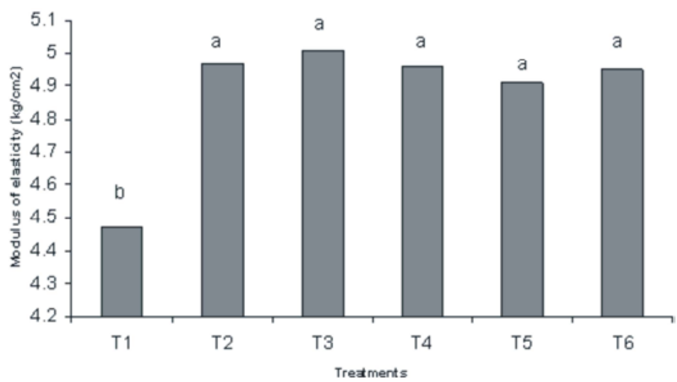

Fig. 2: Effects of feeding antibiotic alternatives on modulus elasticity of tibia bone

T1= c control, T2= control + 15 ppm of Virginiamycin, T3= control + 100 mg probiotic Protexin/kg diet, T4= control + 100 mg prebiotic Immnuwall/kg diet, T5= control + 450 mg commercial herbal blend (Digestrom)/kg diet, T6= control + 400 mg of organic acid (Formycine) /kg diet.

Bars with no common letter differ significantly ($P< 0.05$).

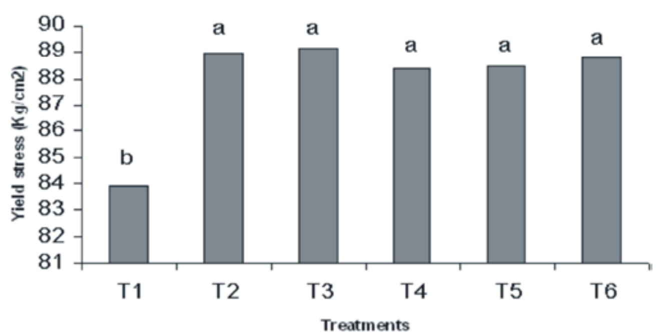

Fig. 3: Effects of feeding antibiotic alternatives on Yield stress of tibia bone.

T1= control, T2= control + 15 ppm of Virginiamycin, T3= control + 100 mg probiotic Protexin/kg diet, T4= control + 100 mg prebiotic Immnuwall/kg diet, T5= control + 450 mg commercial herbal blend Digestrom)/kg diet, T6= control + 400 mg of organic acid (Formycine) /kg diet.

Bars with no common letter differ significantly ($P< 0.05$).

Table 3: The effect of diet on blood calcium and phosphorus of Ross broiler

	Treatment[1]							
Blood parameter	Time (d)	T1	T2	T3	T4	T5	T6	SEM[2]
Calcium (mg/dl)	21	12.89[b]	15.37[a]	12.45[b]	12.37[b]	12.55[b]	13.00[b]	0.63
	28	13.31[d]	15.12[a]	16.00[a]	13.87[cd]	13.37[d]	16.62b[c]	0.38
	35	12.50[b]	17.00[a]	16.87[a]	16.52[a]	16.62[a]	16.32[a]	0.81
	42	13.82[c]	16.11[b]	17.63[a]	16.50[a]	16.50[ab]	16.50[ab]	0.44
Phosphorous (mg/dl)	21	3.46[b]	5.11[a]	4.94[a]	5.00[a]	4.50[ab]	4.17[ab]	0.41
	28	3.17[b]	4.70[a]	5.07[a]	3.12[b]	3.41[b]	4.43[a]	0.24
	35	2.57[b]	4.12[a]	3.63[a]	3.81[a]	3.83[a]	3.82[a]	0.35
	42	2.32[b]	4.69[a]	5.15[a]	4.84[a]	4.93[a]	4.43[a]	0.34

[a, b] Means with different superscripts within the same row differ significantly ($P< 0.05$).

1. T1= control, T2= control + 15 ppm of Virginiamycin, T3= control + 100 mg probiotic Protexin/kg diet, T4= control + 100 mg prebiotic Immnuwall/kg diet, T5= control + 450 mg commercial herbal blend (Digestrom)/kg diet, T6= control + 400 mg of organic acid (Formycine) /kg diet.

2. SEM = standard error of mean

Blood Ca and P: The results of Ca and P blood concentration at different times are presented in Table 3. The use of growth promoters increased the concentration of blood Ca and P particularly at 35 and 42 as compared to control treatment. At 28 days, diet supplementation with antibiotic and probiotic had highest concentration of calcium (15.12 and 16.00 respectively). Phosphor concentration of diets with prebiotic and commercial herbal blend had no different with control diet (P<0.05).

DISCUSSION

Dennine and Sothern [21]reported increasing Ca and P availability through adding Virginiamycin in diet led to an improvement in ash percentage as well as fracture resistance of tibia. When probiotic was added to broilers diet, tibia ash percentage, its resistance to fracture [22] and Ca and P content of tibia increased [19]. Supplementation of a diet with low availability phosphorous and citric acid resulted in an increase of 2% for bone ash and 3%, 3% and 4% for calcium, phosphorous and zinc retention respectively as compared to control diet. These improvement could due to increasing plasma minerals concentrations [23]. Kim *et al*. [24] reported that (increase bioavailability of calcium with using alfalfa, prebiotic and fructo-oligosaccharide in layer diet could lead to increase retention of calcium in bones) bioavailability of calcium increased as supplementation of layer diet with alfalfa, prebiotic and fructo-oligosaccharide is practiced leading to increasing of calcium retention in bones. The percentages of ash and calcium as well as resistance to fracture were significantly (P<0.05) higher compared to control diet. They also found a positive correlation between ash content and fracture resistance. Therefore, it could be concluded that diets supplemented with antibiotic or its replacements may cause an increase of intestinal mineral absorption which in turn this results in increasing of calcium and phosphorous bioavailability for birds.

Index of weight to length of bone was first introduced by Seedor *et al*. [25] (showing significant change of bone mineralization. In fact, this index indicates that bone density so that the higher the index the more dense of the bone [26]. The reason for increasing the index in supplemented diet compared to control diet could be attributed to significant increase of tibia weight. These findings are in contrast to the results of Mutus *et al*. [19] who found that diet supplemented with probiotic had no significant effect on tibiotarsi index. Tibiotarsal index indicates the bone mineralization with using morphological estimates [27]. There is a positive correlation between tibiotarsal index and bone mineralization magnitude in human and dog [28, 29]. A high tibiotarsal index is associated with a high mineralization level of bone [30].

Mutus *et al.* [19] reported that tibiotarsal index for broilers fed diet with BioPlus 2B probiotic was significantly (P<0.05) greater than the control diet suggesting that mineralization degree and bone development is improved for a probiotic supplemented diet. Nahashon *et al*. [31] showed that inclusion of *Lactobacillus* in the diet resulted in a significant (P<0.05) increase of calcium and phosphorous retention due to the fact that these two mineral elements were reserved to a greater extent in egg shell as well as hen's skeleton. Therefore, based upon the measurements of calcium and phosphorous levels in blood (Table 3) and the results of measuring ash, calcium and phosphorous percentages of tibia bone (Figure 1), it can be concluded that supplementation of diet with antibiotic replacements may be caused an increase digestibility and availability of nutrients (such as Ca and P) due to developing desirable micro flora of digestive tract which in turn results in increasing mineral elements retention and bone mineralization.

Mutus *et al.* [19] pointed out that diaphysis diameter was not significantly affected by probiotic supplementation while external and internal diameter of tibia bone and modular diameter showed significant ($P<0.05$) increase and decrease respectively as compared to control diet.

Bone strength is measured as required force (in kg) to break a bone [32]. However, Patterson *et al*. [33] pointed out that yield stress and modular elasticity are more appropriate terms to describe bone strength with respect to different weight and length measures of bones at different bird species. As less dimension of a bone is associated with more resistance to break, bone length is considered as an important factor affecting on breaking. In fact, modulus of elasticity describes bone hardness and its constitutional materials while yield stress is associated with only bone hardness. In other hands, more hardness of a bone is related to a greater value of modular elasticity [34].

Using virginiamycin in diet likely led to increase of calcium and phosphorous availability and resulted in improving ash percentage and resistance to fracture in tibia bone. Virginiamycin could be increased more intestinal nutrients absorption through affecting micro floral protein synthesis of digestive tract [21].

Mutus *et al.* [19] indicated that chickens fed with prebiotic diet had a greater value for modular elasticity along with an increase of ash percentage of tibia bone. Panda *et al*. [22] suggested that *Lactobacillus sporgenes* has a positive effect on tibia ash and also on resistance to fracture possibly due to more retention of calcium in bones. In fact, supplementation of a diet with probiotic (100 and 200 mg per kg) could lead to a more magnitude of serum calcium associating with establishing a more appropriate absorption environment in digestive tract.

Therefore, including virginiamycin, probiotic, prebiotic, medicinal plants or organic acid in diet could result in a significant increase for percentages of ash, Ca and P in tibia bone as compared to control diet and with respect to the results found by Rowland *et al.* [35] a positive association between bone ash p ercentage and its resistance to fracture) this in turn results in increasing bone modular elasticity and its yield stress in chicks fed with supplemented diet. In other words, using antibiotic or its replacements in broiler diet could lead to increase bone fracture resistance due to a higher level of minerals absorption resulted from a better digestibility process.

The result of the present experiment indicated that supplemented diets with virginiamycin and its alternatives had a potential to be added in broiler feed to improve bone characteristics and increase bird heath performance.

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