Research Note

The Effect of Dietary Probiotic Supplementation on Tibial Bone Characteristics and Strength in Broilers

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ABSTRACT A 6-wk study with 50 birds was conducted to investigate the effects of a dietary supplemental probiotic on morphometric parameters and yield stress of the tibia. Twenty-five 1-d-old broiler chicks were assigned to a control or an experimental diet containing *Bacillus licheniformis* and *Bacillus subtilis* (BioPlus 2B, CHR Hansen BioSystems, Denmark, Ugur Ecza Deposu, Turkiye Distributoru, Adapazari 41400, Turkey; each containing 2.3 $\times 10^8$ cfu/g of spores) supplemented to the starter and finisher diets at 500 g/1,000 kg of feed. Each diet was replicated 5 times with 5 birds in each replicate. Tibiotarsi weight, length, and weight/length index, robusticity index, diaphysis diameter, modulus of elasticity, yield stress parameters, and percentage Ca content were not affected by the dietary supplementation of probiotic, whereas thickness of the medial and lateral wall of the tibia, tibiotarsal index, percentage ash, and P content were significantly improved by the probiotic. Medullary canal diameter of the tibia of the birds fed the control diet was significantly greater than that of birds fed the probiotic diet. There was no treatment impact on live performance of the birds throughout the 6-wk feeding trial.

Key words: probiotic, tibia, morphometric index, bone strength, broiler

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INTRODUCTION

Subtherapeutic use of antibiotics in poultry feeds has become undesirable because of the residuals in meat products and development of antibiotic-resistant bacterial populations in humans. In Europe, use of antibiotics as growth-promoting agents for poultry has been banned. Therefore, probiotic use has gained widespread interest since Tortuero's (1973) first attempt at using living bacteria to replace antibiotics in poultry. Although there have been some conflicting studies, probiotics have been shown to improve weight gain and feed conversion rate and to reduce mortality (Jin et al., 1997). Probiotics are selected preparations of beneficial microbials, mainly *Lactobacilli*, *Streptococci*, and *Bacillus* species. Although modes of action are not entirely clear, probiotics are thought to influence the intestinal flora by competitive exclusion and antagonistic activity to pathogenic bacteria for the host (Jin et al., 1997). Bilgili and Moran (1990) studied the influence of a withdrawal feed supplemented with whey and a probiotic on the retention of *Salmonella* in broiler birds 6 wk of age. They found no difference in live performance caused by inclusion of whey or probiotic in broilers. Jin et al. (2000) reported that supplementation of a single strain of *L. acidophilus* or a mixture of 12 *Lactobacillus* strains to a basal diet significantly increased the body weight of broilers after 40 d of feeding. Feed conversion rate was also significantly improved by the inclusion of probiotics. Although not significant, the mixture of 12 *Lactobacilllus* strains reduced the overall mortality rate by 47%. Plavnik and Scott (1980) showed that practical diets supplemented with 2.5 or 5.0% brewer's yeast reduced leg weaknesses in broilers. However, Veltman and Jensen (1981) concluded that the fermentation products did not reduce ammonium chloride-induced tibial dyschondroplasia.

Normal bone development in birds is also influenced by nutritional factors, genetics, gender, and the absolute growth rate. Skeletal disorders are more pronounced in fast-growing broilers. The skeleton not only provides structural support for the bird but also is an important mineral source for metabolic needs. Bone tissue is complex and composed of inorganic substances such as Ca and P, which provide hardness and strength, and organic substances, which give elasticity to bone. A number of invasive (bone ash, breaking strength, weight, and bone volume), and noninvasive methods (ultrasound) exist to

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determine the bone mineralization in poultry (Rao et al., 1993; Onyango et al., 2003). Barnet and Nordin (1960) utilized tibiotarsal index as another morphometric method to describe the degree of bone mineralization. Virtama and Telkka (1962) showed a significant positive correlation between this method and bone mineral content in humans. Reisenfeld (1972) and Seedor et al. (1991) similarly used the robusticity index and the bone weight/bone length index to describe bone mineralization. Patterson et al. (1986) indicated that modulus of elasticity is a better term to use than force in making bone strength comparisons when birds differ in size. The aim of this study was to investigate the effects of a commercial dietary probiotic supplement on the tibial bone characteristics and strength in broilers.

MATERIALS AND METHODS

A total of 50 chicks (Avian × Avian) were weighed individually and assigned to 2 diets (control or probiotic) such that the average weight across dietary treatments was similar with 5 birds per floor pen and 5 replicate pens per treatment. The chicks were raised in floor pens (75 × 75 cm) with wood shavings. The control and experimental pens were separated from each other to avoid horizontal transfer of the probiotic organisms. The control or probiotic diets were based on corn, soybean meal, and wheat. The probiotic was a combination of *Bacillus licheniformis* and *Bacillus subtilis* (each containing 2.3×10^8 cfu/g of spores) and was added to starter and finisher diets at 500 g/1,000 kg of feed but not to control diets. Chicks had free access to a starter diet during the first 3 wk and then to a finisher diet for the following 3 wk. Diets met or exceeded NRC (1994) recommendations except for starter nonphytate P, which was 93% of NRC (Table 1). Experimental diets were analyzed for ash, CP, Ca, and P (Association of Official Analytical Chemists, 1994). Birds were provided with continuous fluorescent lighting and also had free access to water.

All the experimental birds were slaughtered by cutting the carotid arteries with subsequent exsanguination. The left and right tibia of each bird were removed as drumsticks with flesh intact. The drumsticks were labeled and immersed in boiling water (100°C) for 10 min. After cooling to room temperature, the drumsticks were defleshed by hand and the patella was removed. They were then air-dried for 24 h at room temperature. The tibiotarsal length and bone weight were determined. Following morphometric measurements, yield stress and modulus of elasticity were determined by the method described by Kocabağlı (2001). Prior to breaking, each bone was marked at midpoint, and outside diameters were measured perpendicular and parallel to the direction of the applied force using a caliper. After breaking, diameter measurements were made inside and outside the midshaft of the bone, perpendicular and parallel to the direction of the applied force to calculate the area moment of inertia. This term was used together with elastic deformation to determine the stress (kg/cm^2) and modulus of bone elasticity (kg/cm^2; Patterson et al., 1986). The thickness of the medial and lateral walls was measured at the midpoint mark using a dial caliper. Medullary canal diameter was calculated by subtracting the thicknesses of the medial and lateral walls from the diameter at the diaphysis. The bone weight/length index was obtained by dividing the tibia weight by its length (Seedor et al., 1991). The tibiotarsal and the robusticity indexes are determined using the following formulas, respectively:

tibiotarsal index =
diaphysis diameter − medullary canal diameter/
diaphysis diameter × 100
(Barnet and Nordin, 1960);

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

To determine bone ash content, bones were oven-dried at 105°C for 24 h and ashed in a muffle furnace at 600°C for 6 h. The percentage ash was determined relative to dry weight of the tibia.

The data were subjected to 1-way ANOVA as a complete block design using the GLM procedure of SAS (SAS Institute, 1994). The experimental unit was an individual bird for the body weight and morphometric parameters while pen was placed in the error term to test if there was any pen effect on aforementioned parameters. Since feed consumption and conversion was determined on a pen basis, pen was used as the experimental unit for these parameters. The Student-Newman-Keul's multiple comparison procedure (Steel and Torrie, 1980) was utilized to compare the treatment means when the *F*-test was significant ($P < 0.05$).

RESULTS AND DISCUSSION

There was no pen effect within treatments for the 6-wk body weights, morphometric parameters, or mineral content of the tibia. Six-week body weights of broilers in the control and probiotic groups were 2,477 and 2,486 g, respectively, and not statistically different. Similarly, feed consumption and conversion were not affected by the dietary probiotic supplementation (Table 2). Tibiotarsi weight and length, tibiotarsi weight/length index, robusticity index, diaphysis diameter, modulus of elasticity, and yield stress parameters were not affected by the probiotic diet (Table 3). Birds fed the probiotic diet had greater medial and lateral wall thickness of the tibiotarsi, whereas birds fed with the control diet had greater medullary canal diameter. The probiotic diet also resulted in a greater tibiotarsal index than the birds fed the control diet. Tibia ash and P concentrations of broilers fed the probiotic-supplemented diet were significantly greater than those fed with the control diet, whereas the percentage of Ca was not affected by either of the experimental diets.

Table 1. Composition of the experimental broiler diets, % as-is basis

	Starter		Finisher	
Ingredient	Control	Probiotic	Control	Probiotic
Ground corn	56.05	56.00	50.80	50.75
Wheat	2.50	2.50	9.85	9.85
Soybean meal (46% CP)	30.00	30.00	29.00	29.00
Fish meal (67% CP)	5.00	5.00	2.00	2.00
Soybean oil (8,929 kcal/kg)	3.00	3.00	5.00	5.00
Limestone	1.19	1.19	1.20	1.20
Dicalcium phosphate[1]	1.20	1.20	1.15	1.15
Vitamin-mineral premix[2]	0.25	0.25	0.25	0.25
Vitamin E[3]	0.10	0.10	0.10	0.10
Sodium bicarbonate	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25
DL-Methionine	0.16	0.16	0.10	0.10
Coccidiostat	0.10	0.10	0.10	0.10
Ethoxyquin	0.10	0.10	0.10	0.10
BioPlus 2B[4]	—	0.05	—	0.05
Total	100.00	100.00	100.00	100.00
Calculated and analyzed value				
Metabolizable energy, kcal/kg	3,089	3,086	3,216	3,216
CP, % (analyzed)	22.94	22.93	20.94	20.93
Lysine, %	1.31	1.31	1.14	1.14
Methionine, %	0.52	0.52	0.41	0.41
Methionine + cystine, %	0.74	0.74	0.66	0.66
Ca, % (analyzed)	1.04	1.03	0.93	0.92
P, % (analyzed)	0.51	0.50	0.41	0.40
Nonphytate P, % (calculated)	0.42	0.42	0.36	0.36

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

[2]Supplied the following per kilogram of diet: vitamin A, 15,000 IU; cholecalciferol, 1,500 ICU; vitamin E, 30.0 IU (DL-α-tocopheryl acetate); menadione, 5.0 mg; thiamine, 3.0 mg; riboflavin, 6.0 mg; niacin, 20.0 mg; pantothenic acid, 8.0 mg; pyridoxine, 5.0 mg; folic acid, 1.0 mg; vitamin B_{12}, 15 μg; manganese, 80.0 mg; zinc, 60.0 mg; iron, 30.0 mg; copper, 5.0 mg; iodine, 2.0 mg; and selenium, 0.15 mg.

[3]DL-α-Tocopheryl acetate (premix, 50%). DSM Nutritional Products Ltd. CH-4002 Basel, Switzerland.

[4]A combination of *Bacillus licheniformis* and *Bacillus subtilis*, each containing 2.3×10^8 cfu/g of spores.

The results of the present experiment indicate that broilers fed the probiotic-supplemented diet had significantly greater thicknesses of the lateral and medial walls of the tibia and tibiotarsal index (Table 3). Plavnik and Scott (1980) reported that chickens receiving brewer's yeast had much lower incidence of tibial dyschondroplasia, and bone strength was increased by the dietary yeast supplementation.

The bone weight/bone length index is a simple index of bone density obtained by dividing bone weight by its length (Seedor et al., 1991). The higher the index, the denser is the bone (Monteagudo et al., 1997). On the contrary, low robusticity index indicates a strong bone structure (Reisenfeld, 1972). Also the high value of the tibiotarsal index shows the high mineralization level of the bone (Von Hartung and Van Hasselt, 1988). The tibiotarsal index in the probiotic-fed broilers was higher compared with the control-fed birds (Table 3). These results suggest that the supplemental probiotic herein increased the degree of mineralization and development of bone. Nahashon et al. (1994) reported positive correlations between *Lactobacillus* diets (1,100 and 2,200 ppm) and P and Ca retentions, daily feed consumption, and egg size. Dietary *Lactobacillus* supplementations also resulted in better egg mass, egg weight, egg size, and body weight gain compared with the control. Furthermore, they speculated that higher than normal retained Ca and P might have been deposited in the eggshells or the skeleton. The percentages of tibia ash and P content were significantly improved by dietary probiotic supplementation in this study (Table 3). Increased bone ash might suggest an improvement in bone mineralization.

Table 2. Body weight, feed consumption, and feed conversion of birds fed control and probiotic diets from 0 to 6 wk of age[1]

Live performance	Control	Probiotic	SEM	Significance
Body weight, g (6 wk)	2,477	2,486	19	NS
Feed consumption,[2] g (0 to 6 wk)	4,404	4,497	57	NS
Feed:Gain[2], g:g (0 to 6 wk)	1.78	1.78	0.029	NS

[1]Each treatment was represented by 5 birds from each 5 replicate pens.

[2]Feed consumption and feed conversion were measured on a per pen basis; therefore, pen was used as the experimental unit.

NS: $P > 0.05$.

Table 3. The effect of probiotic supplementation on morphometric parameters, mineral content, and bone strength measurements of tibia at 6 wk of age[1]

Parameter	Control	Probiotic	SEM	Significance
Weight, g	7.59	7.77	0.171	NS
Length, cm	9.83	9.76	0.072	NS
Tibiotarsi wt/length index, mg/mm	77.22	79.59	1.694	NS
Robusticity index	5.01	4.94	0.051	NS
Diaphysis diameter, mm	9.64	9.55	0.088	NS
Thickness of the medial wall, mm	1.58[b]	1.75[a]	0.070	*
Thickness of the lateral wall, mm	2.57[b]	3.03[a]	0.147	*
Medullary canal diameter, mm	5.50[a]	4.76[b]	0.219	**
Tibiotarsal index	43.26[b]	49.99[a]	2.110	***
Modulus of elasticity, kg/cm^2	4,487	5,192	340	NS
Yield stress, kg/cm^2	81.56	83.48	5.480	NS
Ash, %	56.79[b]	60.25[a]	0.804	**
Ca, %	22.52	23.63	0.565	NS
P, %	10.06[b]	11.26[a]	0.178	**

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

[1]Each treatment was represented by 5 birds from each 5 replicate pens.

NS: $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

The stress at yield reflects the rigidity of bones as a whole, whereas the slope of the linear region of the stress vs. strain curve (Young's modulus or elastic modulus) reflects the intrinsic stiffness or rigidity and material properties of bone. High modulus values indicate the bone to be more rigid, whereas a low modulus could mean the bone is more ductile (Rath et al., 2000). Although not statistically significant, probiotic supplementation slightly improved tibia yield stress and modulus of elasticity of birds in the present study (Table 3). Furthermore, bone ash percentage is usually positively correlated with bone-breaking strength (Rowland et al., 1967).

Microbial probiotics for poultry have been extensively reviewed by Stavric and Kornegay (1995), Jin et al. (1997), Simmering and Blaut (2001), and Patterson and Burkholder (2003). According to these review articles, it is concluded that there has not been a well-established link between microbial probiotics and mineral absorption, or bone development. The present study demonstrated that morphometric properties of the tibia of broilers are affected when fed probiotic-supplemented diets. However, there is a need for further studies to determine the effects of probiotics on bone development in broilers and the mode of action of probiotics on bone mineralization.

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