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≪Research Note≫

Effect of *Bacillus cereus* and Phytase on the Expression of Musculoskeletal Strength and Gut Health in Japanese Quail (*Coturnix japonica*)

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We conducted a 28-day trial to evaluate the influence of *Bacillus cereus* and phytase supplementation on the expression of musculoskeletal strength and intestinal histological features in Japanese quail. Two-hundred day-old quail chicks were divided into four groups with five replicates ($n=10$): the first group served as a control and was fed only a basal diet (BD); the second group was fed BD + 0.1% *B. cereus*; the third group was fed BD + 0.01% microbial phytase; and the fourth group was fed BD + 0.01% microbial phytase + 0.1% *B. cereus*. Compared to the control, individual and combined supplementation of probiotic and phytase increased ($P<0.05$) the tibial weight, length, outside diameter, and weight of bone ash, but decreased ($P<0.05$) the weight-to-length index. The water-holding capacity, fiber diameter, fiber cross-sectional area, number of fibers per unit area, fascicle diameter, and fascicle cross-sectional area increased significantly ($P<0.05$) in birds fed on the combination of phytase and *B. cereus*. The villus height, width, depth, height-to-crypt depth, and surface area increased significantly ($P<0.05$) in the *B. cereus* and phytase groups on an individual basis. The strength of the musculoskeletal system was fully expressed when *B. cereus* and phytase were given synergistically. However, the histological features of the intestines improved in birds fed on an individual basis.

Key words: intestinal morphometry, Japanese quail, pectoralis muscle, phytase, probiotic, tibia bone

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Introduction

Bone is a remarkable organ that performs many functions and undergoes continuous remodeling throughout life (Hauge *et al*., 2001). The complexity, hardness, and strength of bone are due to inorganic substances such as phosphorus and calcium (Onyango *et al*., 2003). Low mineralization increases the risk of bone fracture. Therefore, there is a positive correlation between increased mineralization and certain factors such as feed intake, feed conversion ratio, bone weight, decrease in endogenous loss, and weight gain. Bone density is directly proportional to bone weight (Monteagudo *et al*., 1997). The pectoralis muscle is important in poultry because it is the propulsive organ that enables flight. It only accounts for 10% of the body weight when feeding and supplementation are inadequate (Roy *et al*., 2006). The health of the small intestinal improves with probiotic supplementation in broiler chickens (Khan and Naz, 2013). Probiotics also influence the intestinal microbial balance (Abudabos *et al*., 2017), adjust the gut pH, and improve digestion and enzymatic action (Khan *et al*., 2016). The microorganisms used as probiotics include various species of genera such as *Lactobacillus*, *Enterococcus*, *Bacillus*, and *Streptococcus*, as well as yeast (Khan and Naz, 2013).

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Phytase is a phosphatase enzyme that catalyzes the breakdown of phytic acid into inorganic phosphate (Abd El-Hack *et al*., 2018). It reportedly strengthens bone and improves nutritional efficiency (Rath *et al*., 2000; Kim and Lei, 2005). Dietary phytase supplementation improves the health of the gut by reducing the levels of gastrointestinal tract secretions, leading to enhanced productivity and utilization of energy (Oduguwa *et al*., 2007). The purpose of the present study was to determine the effect of *Bacillus cereus* and phytase on the bone and muscle strength, and ilium histology of quails.

Materials and Methods

Experimental Design and Bird Husbandry

We weighed 200 day-old quail chicks and randomly divided them into four groups; each group contained 50 birds with five replicates ($n=10$) in a completely randomized design (CRD). The experimental birds were maintained in independent brooding quail sheds under standard management conditions for 10 days. The temperature and humidity on Day 1 were maintained at 34±1.5℃ and 65±5%, respectively, and the temperature was reduced by 3–4℃ per week until it reached 25℃ on Day 21.

Dietary Plan

The birds were fed a starter and finisher commercial corn-based basal diet (BD) supplemented with either probiotic (*B. cereus*) or microbial phytase with *ad libitum* access to fresh water for 28 days. The first group was fed only BD, and served as a control; the second group was fed BD + 0.1% *B. cereus*; the third group was fed BD + 0.01% microbial phytase; and the fourth group was fed BD + 0.01% microbial phytase + 0.1% *B. cereus*. The composition of the basal feed is given in Table 1.

Table 1. **Ingredients (%) and composition of the basal diet**

Ingredients	CP 23%
Maize	35.13
Wheat bran	1.00
Canola meal	15.00
Rapeseed meal	4.00
Soybean meal	15.65
Corn gluten meal	1.20
Poultry byproduct meal	0.00
Fish meal	2.00
Marble chips	0.80
DCP	0.80
Lysine sulphate	0.57
DL methionine	0.09
Threonine	0.06
Molasses	0.63
Premix[1]	0.24
Salt	0.18
Rice broken	22.60

[1] Vitamin–mineral premix contains the following per kg: vitamin A, 2400000 IU; vitamin D, 1000000 IU; vitamin E, 16000 IU; vitamin K, 800 mg; vitamin B_1, 600 mg; vitamin B_2, 1600 mg; vitamin B_6, 1000 mg; vitamin B_{12}, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000 mg; iodine, 400; iron, 1200 mg; manganese, 18000 mg; selenium, 60 mg; and zinc, 14000 mg.

Sample Collection and Processing

Two birds from each replicate were slaughtered on Day 28. The left and right tibias of each quail were detached as drumsticks. The drumsticks were labeled and boiled in water at 100℃ for 10 min, then cooled to room temperature. The flesh on the drumsticks was removed and the bones were air-dried at room temperature for 24 h. The visceral organs such as the liver, spleen, small intestine, and cecal tonsils were collected. We obtained 5-cm long sections from the ileum to investigate its histomorphology. Tissue samples for muscle and intestinal histology were fixed in neutral buffered formalin (10%) immediately after slaughter. The tissues were dehydrated by transferring them through a series of alcohol solutions with increasing concentrations, then embedded in paraffin. We obtained 5 μm-thick sections by microtomy, and stained them with hematoxylin and eosin, as described by Tufail *et al*. (2019). All the slides were examined under a light microscope (LABOMED®, USA) supplied with a camera connected to a computer with Prog Res® 2.1.1 Capture Prog Camera Control Software.

Analysis of the Tibial Bone

We measured the length and weight of each tibiotarsal. The outside diameter was measured using a digital caliper by marking the bone at the mid-point. The diameter of the medullary canal was measured with a digital caliper after breaking the bone at the middle position. The bone weight/length index was determined by dividing the bone weight by its length. We dried each defatted and dry tibia bone in a hot air oven for 24 h. After drying, we crushed the bones to a powder. To estimate the mass of the bone ash, we placed 2-g samples of bone in a crucible and burnt them in a muffle furnace at 560℃ for 24 h. We then cooled the samples and weighed the bone ash. The percentage of tibia bone ash was calculated relative to the dry weight of the tibia bone (Rath *et al*., 2000).

Analysis of the Pectoralis Muscle

The muscle pH was measured using a digital pH meter (Young *et al*., 2004). We made a deep vertical incision in the pectoralis muscle after 5 h, and following calibration inserted a pH meter probe 1 cm into the incision. The water-holding capacity (WHC) was measured by pressing a 1-g meat cube between two glass plates by applying an 8–10 kg weight for 5 min. We measured the weight of the pectoralis muscle before and after the procedure, and the difference in weight represented the water loss (Pelicano *et al*., 2003). For the investigation of the histomorphometry of the pectoralis muscle, the diameter of the muscle fiber and fascicle were calibrated using the Progress Capture Pro 2.7.7 program (Labomed, USA), and the muscle was examined under a bright field microscope. The number of muscle fibers/unit area and the number of muscle fascicles/unit area were calculated randomly.

Histomorphometry of the Ilium

The villus height was measured from the top of the villus to the villus–crypt junction, and the crypt depth was measured from the depth of the invagination between the adjacent villi (Chand *et al*., 2019). The surface area of the villus was calculated using the formula$=(2\pi)$ (VW/2) (VL), where VL is the villus height and VW is the villus width.

Statistical Analysis

We carried out the statistical analyses with a statistical package for social science (SPSS Inc. version 20; Chicago IL, USA). The data were analyzed by one-way analysis of variance (ANOVA), and the means were compared using the Tukey test at a significance level of 5%.

Results

The effects of the dietary treatments on the bone quality are recorded in Table 2. The results show that the mean weight, length, outer diameter, and ash content were significantly ($P<0.05$) high in quails fed with a combination of phytase and probiotic. However, the weight-to-length index was significantly ($P<0.05$) reduced in the same group. Overall, we observed improved tibial characteristics either alone or in combination compared to the control.

The effects of the dietary probiotic and phytase on the various characteristics of the pectoralis muscle are reported in Table 3. There was no difference in the pH of the pectoralis muscles between the groups. The water-holding capacity of the pectoralis muscles increased significantly ($P<0.05$) in the phytase + probiotic group. The fiber diameter, fascicle diameter, cross-sectional area of the fibers, and fascicle diameter increased significantly ($P<0.05$) in the phytase groups (either phytase alone or in combination with the

Table 2. **Influence of probiotic and phytase supplementation on gross characteristics of tibia bone in Japanese quail**

Parameters	Control	*Bacillus cereus*	Phytase	*Bacillus cereus* + Phytase
Weight (mg)	356.40 ± 8.11^b	371.8 ± 7.45^a	368.4 ± 6.54^a	374.8 ± 8.21^a
Length (mm)	38.53 ± 4.37^b	47.73 ± 4.12^a	45.69 ± 2.74^a	48.16 ± 3.23^a
Outside diameter (mm)	2.50 ± 0.68^b	2.73 ± 0.24^a	2.70 ± 0.51^a	2.78 ± 0.31^a
Medullary canal diameter (mm)	1.08 ± 0.16	1.13 ± 0.34	1.10 ± 0.21	1.19 ± 0.12
Wall thickness (mm)	0.71 ± 0.16	0.80 ± 0.19	0.79 ± 0.19	0.79 ± 0.10
Weight/ length index	9.38 ± 1.46^a	8.00 ± 1.67^b	8.06 ± 1.45^b	8.02 ± 1.34^b
Ash%	40.32 ± 1.22^d	50.57 ± 1.74^b	43.71 ± 1.92^c	53.93 ± 1.73^a

Values in each row with different superscripts differ significantly ($P<0.05$).

Table 3. **Characteristics of the pectoralis muscle in the control, and in the phytase- and probiotic-supplemented Japanese quail**

Characteristics	Control	*Bacillus cereus*	Phytase	*Bacillus cereus* + Phytase
pH	6.21 ± 0.14	6.19 ± 0.19	6.22 ± 0.04	6.23 ± 0.01
Water-holding capacity (%)	69 ± 2.15^b	67 ± 3.52^b	65 ± 2.48^c	72 ± 2.43^a
Fiber diameter (μm)	12.65 ± 1.47^c	14.93 ± 2.38^b	16.75 ± 1.12^a	16.40 ± 1.26^a
Fiber cross-sectional area (μm)^2	125.94 ± 3.57^c	178.8 ± 17.14^b	220.89 ± 7.89^a	212.49 ± 10.12^a
No. of fibers/unit area (0.01 mm^2)	42 ± 0.32^a	29 ± 0.13^b	30 ± 0.34^b	26 ± 0.78^c
Fascicle diameter (μm)	313.87 ± 34.14^c	371.71 ± 19.56^b	448.75 ± 37.17^a	442.75 ± 14.49^a
Fascicle cross-sectional area (μm^2)	78157 ± 5336^c	108739 ± 3634^b	159081 ± 3536^a	154027 ± 3453^a
No. of fascicles/unit area (0.01 mm^2)	11 ± 0.16^a	9 ± 0.11^b	7 ± 0.18^c	7 ± 0.66^c

Values in each row with different superscripts differ significantly ($P<0.05$).

Table 4. **Influence of the probiotic and phytase on the morphometric characteristics of the ilium in the Japanese quail**

	Control	*Bacillus cereus*	Phytase	*Bacillus cereus* + Phytase
Villus height (mm)	0.34 ± 0.1^{a}	0.33 ± 0.1^{a}	0.23 ± 0.1^{b}	0.22 ± 0.1^{b}
Villus width (mm)	0.07 ± 0.01^{c}	0.09 ± 0.01^{b}	0.11 ± 0.01^{a}	0.08 ± 0.01^{bc}
Crypt depth (mm)	0.08 ± 0.1^{b}	0.14 ± 0.1^{a}	0.15 ± 0.1^{a}	0.10 ± 0.01^{b}
Villus height: crypt depth	3.07 ± 0.1	2.39 ± 0.1	1.80 ± 0.1	2.23 ± 0.1
Villus surface area (mm^2)	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.06 ± 0.01

Within the same row, means with different superscripts are significantly different ($P < 0.05$).

probiotic) compared to the control. The number of fibers per unit area and the number of fascicles per unit area decreased significantly ($P < 0.05$) in the phytase groups (either phytase alone or in combination with the probiotic).

The effects of the probiotic and phytase on the morphometric parameters of the ilium in Japanese quails are reported in Table 4. The results show that the villus height decreased significantly ($P < 0.05$) in the phytase- and probiotic-supplemented groups compared to the control. The villus height increased significantly ($P < 0.05$) in the phytase-supplemented group. The crypt depth increased significantly ($P < 0.05$) in the probiotic- and phytase-supplemented groups on an individual basis compared to the control and their combination.

Discussion

The tibia is very important for locomotion and weight-bearing in birds, and it strengthens the musculoskeletal system. In the current study, most of the bone quality parameters were improved in the birds fed with a combination of phytase and the probiotic. Our findings are partially corroborated by those of Viveros *et al*. (2002). However, they are contradicted by those of Mutus *et al*. (2006) and Zhou *et al*. (2008). The ash content increased significantly ($P < 0.05$) in the group co-supplemented with the probiotic and phytase. The results of the current study agree with those of Viveros *et al*. (2002) and Mutus *et al*. (2006). The phenomenon could be due to the increased mineral absorption from the intestines. The improved bone characteristics of the tibia in the current study may have been due to the anti-stressor properties of the probiotic and phytase. Skeletal disorders are well known in birds, and the results of various studies suggest that probiotics strengthen the bone in fast-growing birds (Khan and Naz, 2013). The quality of the bone was improved in the present study, possibly owing to the positive effect of the probiotic and phytase.

In the current study, most of the characteristics of the pectoralis muscle fiber improved significantly in the diet-supplemented quails. The results are in agreement with those of Maiorano *et al*. (2012). Literature on the effects of probiotics and phytase on most of the characteristics of muscles is scarce. The increased muscle fascicle diameter and cross-sectional area may be due in part to the higher relevant muscle fiber and area. It is also possible that the increased diameter of the muscle may be due to the muscle growth-promoting action of the phytase and probiotic bacteria.

In the ileum, dietary supplementation with phytase and its combination with the probiotic decreased the villus height compared to the control group. The findings with regard to the villus height in the ileum agreed with those of Burkholder *et al*. (2008). The villus width and crypt depth were higher ($P < 0.05$) in the phytase group than in the control group. The results of the current study relate to some previous studies (Pirgozliev *et al*., 2007; Shah *et al*., 2019). The improved gut health observed in the present study may have been due to the proliferation of the ilium cells. Shah *et al*. (2019) reported that dietary probiotic supplementation improved the morphometry of the intestines by increasing the villus height and crypt depth.

From the results of the present study, we conclude that supplementation with *B. cereus* and phytase, either individually or in combination, improves the health of the gut and musculoskeletal system. However, the best results were observed when *B. cereus* and phytase were used in combination.

Conflicts of Interest

The authors declare no conflicts of interest.

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