Influence of Phytase on Tibia Bone Characteristics of Broiler Quail Fed on Corn-Soybean Meal Diets

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Abstract:

BACKGROUND: Exogenous phytase enhances the utilization of plant phytate phosphorus in poultry. **OBJECTIVES:** In the present study the effects of exogenous phytase was investigated on tibia bone characteristics of white quail. **METHODS:** In a 2x2 factorial arrangement, eighty, 11-day old unsexed chicks were randomly divided into 4 treatments of 20 replicates. All birds received one of four experimental diets with two levels of aP (5.2 or 3.9 g kg^{-1}) and two levels of phytase (0 or 500 FTU kg^{-1}). On day 25, all birds were weighed, sacrificed, and blood samples were obtained for the determination of blood phosphorus. Both tibiae were excised for the determination of ash, calcium and phosphorus content, bone length and thickness, and breaking strength. **RESULTS:** Birds which had received lower aP diets showed lower levels of blood phosphorus as well as tibia bone weight, ash, calcium, phosphorus, bone thickness and strength when compared with the higher aP fed birds. **CONCLUSIONS:** Adding phytase to the lower aP diet was able to restore the above-mentioned variables. Phytase increases rigidity and strength of tibia by enhancing the availability of phytate phosphorus to the quail. This is important, particularly when the bird is grown for flight and hunting.

Introduction

Differences exist in availability of dietary plant and mineral phosphorus between poultry species (Rodehutscord and Dieckmann, 2005). In most cereal grains and oilseeds the phytate P is the main storage form of phosphorus and represents 50 to 85% of the total P. Quail, like other poultry species, poorly utilize phytate P and this affects the proper digestion of dietary nutrients, increases the cost of diet and, more importantly, promotes environmental pollution (Jongbloed and Lenis, 1998; Ravindran et al., 2000; Selle et al., 2000; Adeola and Sands, 2003). Phytase is an enzyme that hydrolyzes phytate to inositol and inorganic phosphate. Since there is no natural phytase in the gastro-intestinal tract of poultry, supplementation of diets with exogenous phytase has been proven to enhance the digestibility of phytate P (Nys et al., 1999). Phytase reduces the need for supplemental inorganic P to the diet and consequently, the amount of P excreted into the environment (Keshavarz, 2000; Selle et al., 2000; Kies et al., 2001; Adeola and Sands, 2003; Yu et al., 2004). Although a number of studies have demonstrated the positive influence of microbial phytase on the digestibility of dietary phytate phosphorous and other nutrients in Japanese Quail (*Coturnix coturnix Japonica*) (Bahtiyarca and Parlat, 1997; Saricicek et al., 2005; Vali, 2010; Lima et al., 2011 $_{a,b}$), there is a paucity of evidence on the effect of phytase on growth and development of bone in quail.

To evaluate the effect of phytase on bone mineralization in white quail, blood P level and tibia bone characteristics of young quail fed with corn and soybean meal based diets with two levels of available P (aP) with and without phytase were compared.

Materials and Methods

The experiment was carried out at the Poultry Station, Veterinary Research Institute, Faculty of Veterinary Medicine, University of Tehran, Tehran - Iran. The experimental procedure was approved by the Animal Research Committee of the University of Tehran. A total of 130 one-day old mixed white quail chicks were housed in a wire cage with newspaper litter in an environmentally controlled room, with 23 h constant lighting. Chicks received a commercial starter diet containing 2750 kcal kg^{-1} metabolisable energy, 270 g kg^{-1} crude protein, 12 g kg^{-1} total calcium and 5.2 g kg^{-1} aP, until 11 days of age. In a 2×2 factorial arrangement, eighty individually identified, unsexed birds with similar body weights (55±0.8 g), were randomly assigned into 4 wire cages (90 × 60 × 40 cm). Each cage contained 20 birds and each bird was regarded as one experimental unit (replicate). All birds received one of four iso-energitic, iso-nitrogenous corn-soybean meal based experimental diets which were formulated to meet or exceed the nutritional requirements of quail as established by the NRC (1994) (Table 1). Diets were arranged to contain two levels of aP (Treatments A and B=5.2 g kg^{-1}, Treatments C and D=3.9 g kg^{-1}) and two levels of a commercially available phytase, Natuphos 5000®G (Treatments A and C = 0 FTU kg^{-1}, Treatments B and D=500 FTU kg^{-1}) (BASF SE, Lampertheim, Germany). One FTU (phytase enzyme unit) is equivalent to the amount of enzyme that liberates micromole inorganic phosphorus per minute from 0.0051 mol/L sodium phytate at 37°C and at pH = 5.5 (Hall et al., 2003). Birds had free access to experimental diets in mash form and fresh water until 25 days of age. The experiment lasted two weeks and on the last day of the experimental period, all birds were individually weighed and the final body weight was recorded. Then all birds were sacrificed by cutting the carotid arteries and following exsanguinations. Blood samples were obtained for subsequent determination of phosphorus in blood (BP) by photometric system. Left tibia was excised, defatted, dried and ashed for the determination of tibia weight (TW) and tibia ash weight (TAW). Calcium and phosphorus contents of the ash samples were determined using volumetric (Code 935.13) and spectrophotometric methods (Code: 964.06), respectively, according to the AOAC (http://aoac.org/). Right tibia was used for bone geometrical parameters including bone length (BL) and width (BWi) using a micrometer. The ratio of bone width to bone length (BWi/BL) was calculated. The tibia weight/length index (TW/BL) was determined by dividing the bone weight by its length (Seedor et al., 1991). The Robusticity Index was also calculated by dividing the bone length by the cube root of tibia weight (Mutus et al., 2006).

Bone-breaking strength (BBS) analysis was also conducted on right tibia using an Instron Materials Tester (Model 4486, Instron Corp., Canton, MA), according to the method described by Jendral et al. (2008). Briefly, each bone was placed on 2 support points measuring 2.5 cm apart. A shear plate, 80 mm in length and 1 mm wide, attached to a 50-kg load cell with a crosshead speed of 20 mm/min, was introduced to the midpoint of the same facial plane of each bone and the breaking strength was recorded in Newton.

Analysis of response variables between each treatment was carried out using one-way analysis of variance (ANOVA) of Minitab system (Minitab 13.2 statistical package, Minitab Inc. State College, 2000). Fisher's LSD procedure was used to obtain confidence intervals for all pair wise differences between means. General linear model (GLM) procedure was used to determine the main effects of factors (aP level, Phytase, aP level × Phytase) on the response variables. Statements of statistical significance are based on a probability of $p<0.05$.

Results

Although no statistical analysis was carried out on body weight gain of the birds, there was no notable difference in body weight gain among the experimental treatments as the mean body weight gain of treatments A, B, C and D were 112, 109, 107 and 111 ±1.4 g, respectively. Those birds fed on lower aP diet without phytase had the lowest BP among the experimental groups (Table 2). However, adding the enzyme restored the BP of these birds. The experimental birds fed on lower aP diet without phytase also had the lowest TW, TAW, TCaW, TPW, BBS and BWi. The same birds showed the lowest

Table 1. Feed ingredients and calculated nutritional composition of experimental diets for broiler quail from 11 to 25 days of age. *Vitamin and mineral premix supplied per kg of diet; Retinol acetate 24 mg, Cholecalciferol 24mg, Tocopherol acetate 30mg, Thiamine 1.8mg, Riboflavine 6.6mg, Calcium panthotente 10mg, Nicotinic acid 30mg, Pyridoxine hydrochloride 3mg, Cyanocobalamin 0.015mg, Biotin 0.1mg, Folic Acid 1.0mg, Choline Chloride 500mg, Menadione 2mg, Manganese 99.2mg, Iron 50mg, Zinc 84.7mg, Copper 10mg, Iodine 1mg, Selenium 0.2mg.

Feed Ingredients	Adequate Diet (g kg-1)	Marginal Diet (g kg-1)
Corn	**492**	**492**
Soya Meal (44%)	**440**	**440**
Vegetable Oil	**20**	**20**
Calcium Carbonate	**16**	**20**
Vit-Min Premix*	**4**	**4**
Di-Calcium Phosphate (18% P)	**19**	**13**
DL-Methionine	**2**	**2**
L-Lysine	**1.5**	**1.5**
Salt	**4**	**4**
Sand	**0**	**2**
Zinc Bacitracin	**0.5**	**0.5**
Choline Chloride (60%)	**1**	**1**
Nutritional Composition		
Metabolizable Energy (kcal kg-1)	**2810**	**2810**
Crude Protein	**233**	**233**
Crude Fat	**42.1**	**42.1**
Crude Fiber	**41.5**	**41.5**
Total Calcium	**12.0**	**12.0**
Total Phosphorus	**7.8**	**6.5**
Available Phosphorus	**5.2**	**3.9**
Total Sodium	**2.0**	**2.0**
Methionine	**5.6**	**5.6**
Lysine	**14.3**	**14.3**
Methionine+Cystine	**9.4**	**9.4**

ratio of BWi/BL and TW/BL but the highest ratio of Robusticity Index, as well. The addition of phytase enzyme, particularly to the lower aP diet increased TW, TCaW, TPW, BBS, BL and BWi. The ratio of BWi/BL and TW/BL Index were also improved by the enzyme. Interactions were observed between dietary aP level and phytase enzyme in TW, TCaW, TPW, BB S, BWi and BWi/BL.

Discussion

Based on the recommendations of NRC (1994), dietary Ca and aP requirements of quail at starting and growing stages are 8 and 3 g kg $^{-1}$, respectively, with the ratio of Ca/aP = 2.66. However, Costa et al. (2011) recommended that the dietary Ca level for broiler quail from 1 to 21 days of age could be set at 12 g kg^{-1}. In our experiment, in order to evaluate the influence of phytase enzyme on the characteris-tics of tibia bone, the amount of total calcium in all experimental diets was kept constant at the level of 12 g kg $^{-1}$ and the amount of aP was set at two levels of 5.2 (ratio of Ca/aP = 2.30) and 3.9 g kg $^{-1}$ of diet (ratio of Ca/aP = 3.07), respectively. As a result, the chosen dietary ratios of Ca/aP in this experiment were 15% higher or 15% lower than recommended Ca/aP ratio by NRC (1994). This experiment did not aim to evaluate the influence of aP level and/or phytase on body weight gain, feed intake and feed conversion ratio of the birds, as it is frequently reported that diets with aP levels much lower than the aP levels used in this experiment had no negative influence on growth performance of broiler quail (Sacakli et al., 2006; Alfotieh and Bessei, 2008; Vali, 2010). Sacakli et al. (2006) showed that reducing dietary available P from 3.5 g kg $^{-1}$ to 2.0 g kg $^{-1}$ had no effect on BWG, feed intake and FCR of quail. Alfotieh and Bessei (2008) reported that diets containing very low amounts of available phosphorus fulfilled the requirements of quails and had no effect on BWG and FCR.

A decrease in BP of the birds fed on a diet with the lower level of aP when compared with BP of higher aP fed birds, and an elevation in this variable because of adding phytase to the low aP diet has also been demonstrated in quail as well as in other poultry birds (Fernandes et al., 1999; Brenes et al., 2003; Juanpere et al., 2004; Wu et al., 2004; Sheikhlar et al., 2009; Vali, 2010, Akyurek et al., 2011).

The above result indicates that the added enzyme enhanced the bioavailability of dietary phytate P in the intestinal lumen of the bird by releasing phosphate group form phytate molecule. Phosphate group was then absorbed and increased the blood phosphorous level.

Lower mean values for TW, RTW, TAW, RTCaW, RTPW, BBS, BWi and BWi/BL in birds fed on lower aP diet when compared with the birds fed on higher aP diet, and an enhancement in the above variables with the aid of phytase, are in agreement with previous reports.

Bahtiyarca and Parlat (1997) showed that phytase supplementation at 0.1% level of diets containing

Table 2. Influence of adding phytase to corn-soybean meal diets with two levels of available phosphorus (aP) on blood phosphorous level and tibia characteristics of broiler quail from 11 to 25 days of age. a,b,c, Variable means with different superscripts in each row are statistically different (n = 20, p<0.05); NS, Not significant; *, p<0.05;**, p<0.01; ***, p<0.001; SEM, Standard error of mean. BP, Blood phosphorus; TW, Tibia weight; TAW, Tibia ash weight; TCaW, Tibia calcium weight; TPW, Tibia phosphorus weight; BBS, Bone breaking strength (Newton); BL, Bone length; BWi, Bone width; BWi/BL, Bone width / bone length ratio; TW/BL, Tibia weight / Bone length index.

	5.2 g kg^{-1} aP		3.9 g kg^{-1} aP		SEM	Statistical Significance		
	0 FTU Enzyme	500 FTU Enzyme	0 FTU Enzyme	500 FTU Enzyme		aP Level	Phytase	aP Level ×Phytase
BP (mg/dl)	5.58^{bc}	5.04^{ab}	4.64^{a}	6.00^{c}	0.28	*	*	NS
TW (mg)	515^{b}	520^{b}	473^{a}	510^{b}	12	*	*	*
TAW (mg)	249^{b}	235^{ab}	223^{a}	234^{ab}	6.9	*	NS	NS
TCaW (mg)	91.5^{b}	92.2^{b}	76.9^{a}	91.1^{b}	3.20	*	*	*
TPW (mg)	47.7^{ab}	50.0^{b}	43.8^{a}	48.3^{b}	1.43	*	*	*
BBS (N)	61^{b}	60^{b}	54^{a}	60^{b}	1.9	*	*	*
BL (mm)	53.6^{a}	54.4^{ab}	54.1^{ab}	55.1^{b}	0.41	NS	*	NS
BWi (mm)	2.90^{b}	3.18^{c}	2.73^{a}	3.07^{bc}	0.07	*	**	*
BWi/BL (%)	5.41^{b}	5.83^{c}	5.03^{a}	5.56^{b}	0.11	***	***	***
TW/BL (mg/mm)	9.64^{b}	9.55^{b}	8.72^{a}	9.31^{b}	0.21	***	*	NS
Robusticity Index	6.69^{a}	6.76^{a}	6.99^{b}	6.94^{b}	0.07	***	NS	NS

0.45 and 0.35% aP significantly improved tibia ash and breaking strength in comparison with control group fed 0.45% aP without phytase. Sacakli et al., 2006 noted that tibia ash was reduced for the quails fed diet low in aP compared to the adequate diet. However, the addition of phytase to the diet containing low-level of aP improved tibia ash.

In contrast, Osman et al., 2009 were not able to show the influence of dietary P level or phytase on tibia ash of the birds in their experiment. However they reported that tibia relative weight decreased as a result of decreasing the dietary aP level.

An increase in calcium and phosphorus content of the tibia bone, particularly in birds fed with lower aP diet in response to the addition of phytase, is a clear indication of an effective degradation of phytate phosphorus in the GI tract of the bird by the enzyme and hence an enhanced absorption and utilization of phosphate groups for bone mineralization.

Osman et al., 2009 showed that tibia length of quail chicks was significantly increased as a result of adding microbial phytase to low aP diets. In their experiment, in response to the added phytase, tibia breaking strength of the same birds was also significantly increased.

In our experiment phytase was not only able to increase the length of tibia bone, but also increased the tibia width in both high and low aP fed birds. This helps tibia bone towards a higher rigidity and strength. An 11.3% reduction in BBS of the birds that received lower dietary aP in this experiment and restored BBS of the same birds when the diet contained phytase (11.1%), supports the above-mentioned findings. 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 the bone. In contrast, the low Robusticity Index indicates a strong bone structure (Mutus et al., 2006). A notable reduction in the TW/BL index (≈10.5%) and an elevation in Robusticity Index (≈4.5%) of the lower aP fed birds without phytase in comparison with higher aP fed birds confirms the BBS results and is a clear indication of that the level of dietary aP has a significant influence on the bone structure and rigidity. Although phytase appeared to have no impact on both indices in higher aP fed birds, it was successful in increasing TW/BL Index of lower aP fed birds up to 6.7%.

In conclusion, this study showed that adding phytase enzyme to corn-soybean meal diets improves the characteristics of the tibia bone in quail chicks. Phytase supplementation not only increases the bioavailability of dietary phytate phosphorus, but may also increase the utilization of other required dietary nutrients for the bird and subsequently increase the bone mineralization. This leads to increasing the bone rigidity in quail, particularly when the bird is grown for flight or hunting purposes.

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مجله طب دامی ایران، ۱۳۹۱، دوره ۶، شماره ۳، ۱۵۴-۱۴۹

تاثیر فیتاز بر خصوصیات استخوان درشت نی جوجه‌های بلدرچین تغذیه شده با جیره‌های ذرت ـ کنجاله سویا

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چکیده

زمینه مطالعه: انزیم‌های فیتاز با منشاء خارجی سبب بهبود بکارگیری فسفر فیتاته گیاهی در طیور می‌شوند. **هدف:** در مطالعه حاضر، اثرات فیتاز با منشاء خارجی بر ویژگی‌های استخوان درشت نی بلدرچین‌های سفید مورد بررسی قرار گرفت. **روش کار:** تعداد ۸۰ قطعه جوجه بلدرچین ۱۱ روزه تعیین جنسیت نشده به صورت تصادفی و باآرایش فاکتوریل ۲ × ۲ به چهار تیمار و هر تیمار شامل ۲۰ تکرار تقسیم شدند. تمام پرندگان یکی از چهار جیره‌آزمایشی با دوسطح فسفر قابل دسترس (۵/۲g/kg و ۳/۹) و دوسطح فیتاز (FTU/kg ۵۰۰ و ۰) را دریافت کردند. در ۲۵ روزگی، تمام پرندگان توزین وذبح‌شده و نمونه‌های خون برای تعیین غلظت فسفر خون اخذ گردیدند. هر دو استخوان درشت نی به منظور تعیین مقادیر خاکستر، کلسیم و فسفر، طول، ضخامت و قدرت شکنندگی استخوان قطع و خارج گردیدند. **نتایج:** پرندگان دریافت کننده جیره‌های با فسفر قابل استفاده پائین در مقایسه با پرندگان تغذیه شده با سطوح بالاتر فسفر قابل دسترس، مقادیر پائین‌تری از فسفر خون، و همچنین وزن، خاکستر، کلسیم، فسفر، ضخامت و قدرت شکنندگی استخوان درشت نی را نشان دادند. **نتیجه‌گیری نهایی:** افزودن فیتاز به جیره‌های با سطوح پائین‌تر فسفر قابل دسترس موجب بازگرداندن متغیرهای فوق شد. فیتاز از طریق بالا بردن قابلیت دسترسی فسفر فیتاته، استحکام و مقاومت استخوان درشت نی را افزایش می دهد.

واژه‌های کلیدی: فسفرفابل دسترس، فیتاز، خصوصیات استخوانی، بلدرچین.

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