◀Research Note▶

Evaluation of Commercial Phytases on Performance and Skeletal Strength of Two Broiler Strains

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An experiment was conducted to evaluate the effects of two commercial phytases on performance and bone mineralization of male broilers from two commercial strains when included into a diet marginal in both calcium (Ca) and non-phytate phosphorus (npP). Male chicks from two broiler strains (Strains 1 and 2; 320 of each) were placed on fresh bedding across 64 pens (10 birds/pen with 8 replicates/treatment). All birds were fed a corn-soybean meal diet (22% CP, 3,086 kcal/kg) adequate in all nutrients except Ca and npP. Dietary treatments were arranged as a 2×4 factorial using 2 npP/Ca combinations and four dietary treatments: 1) standard (0.45% npP, 1.0% Ca), 2) marginal (0.35% npP, 0.87% Ca), 3) marginal + 500 FTU Phytase A, and 4) marginal + 500 FTU Phytase B. All diets were pelleted at 79°C, crumbled and provided from 0 to 21 days of age. Individual body weights and feed consumption were recorded at 21 d of age and 24 birds per treatment were sacrificed for tibia breaking strength.

Chicks of Strain 2 were 1.7 g heavier ($P < 0.05$) than Strain 1 chicks at hatch, however there were no significant differences ($P > 0.05$) in body weight at 21 d of age. Body weight, body weight gain, feed consumption and bone strength were significantly decreased ($P < 0.05$) in the marginal diet-fed birds in comparison with the standard diet-fed birds. However, all these parameters were completely reversed with supplementation of either Phytase A or B. Overall, live performance, bone breaking strength, and response to phytase supplementation were not affected by genetic strain or type of phytase utilized in the diet. These findings suggest that reducing dietary npP requires phytase supplementation to obtain normal growth performance and skeletal growth, regardless of commercial broiler strain.

Key words: bone strength, broiler strain, phytase

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Introduction

Inorganic phosphorus (P) sources are needed in the broiler diet due to the presence of phytic acid, a natural storage form of P in many plants (NRC, 1994). As with other monogastric animals, the chicken lacks the ability to break down this molecule in large quantities, resulting in much of the bound-P being underutilized and ultimately excreted (Nahm, 2007). The high costs associated with inorganic P sources, which are traditionally included into a broiler diet to meet the birds' P requirement, has often led to the inclusion of marginal levels of P in the diet. Failure to meet the birds' dietary P requirement can lead to reductions in performance, abnormal skeletal growth and increased mortality (Waldroup, 1999). Phytase is capable of initiating the release of the phytic acid-bound P and are often included into the diet of broiler chickens, enabling inorganic P usage to be decreased in the diet without negatively impacting bird performance.

Within the literature, research has lead to the suggestion that diets supplemented with phytase should be formulated with proper reductions in non-phytate phosphorus (npP) and calcium (Ca). Failure to reduce npP from standard levels in diets supplemented with phytase may result in decreased hydrolysis of the phytic acid molecule (Ballam *et al.*, 1984; Karim, 2006) by inhibiting the catalytic activity of phytase within the digestive tract (Wise, 1983), resulting in decreased absorption of P. High dietary concentrations of Ca also inhibit the hydrolysis of phytate due to the formation of insoluble Ca-phytate complexes (Sands *et al.*, 2003; Tamin *et al.*, 2004), suggesting that phytate hydrolysis is inversely related to dietary Ca levels (Singh, 2008). Furthermore, high Ca: npP ratios suppress the absorption of inorganic soluble forms of P due to increased precipitation of insoluble Ca: P complexes (Hurwitz and Bar, 1971). Thus, appropriate content of Ca and P should be included into broiler diets supplemented with phytase.

Bone strength is influenced by a number of variables. Bone density is a heritable trait in animals (Boskey *et al.*, 1999). Williams *et al.* (2000a, b) have shown that continued selection for faster growth rate can result in greater

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cortical porosity and can increase the Ca: P ratio in bone mineral of broilers, resulting in weaker bones. A number of investigators have reported differences in bone strength between birds having slow and rapid growth rates (Pitsillides *et al.*, 1999; Williams *et al.*, 2000a), though few studies have compared commercial broiler strains. Yalcin *et al.* (2001) found no effect on bone strength for two commercial broiler strains beyond two weeks of age. With the exception of this study, little research has been published to show whether bone mineralization, which is directly related to bone strength, differs between commercial broiler strains.

The objective of the present study was to investigate the effects of two commercial phytases on performance and bone strength of male broilers from two different commercial strains when these two enzymes are included into a diet with appropriate reductions in Ca: npP ratio for enzyme inclusion but marginal in both calcium and npP according to the NRC (1994).

Materials and Methods

Animals and Procedures

A total of 640 male chicks (320 of each strain^{1,2}) from 38 wk-old hens were obtained from a commercial hatchery, vaccinated for Marek's disease, spray vaccinated with Coccivac DTM, and randomly allotted to one of eight treatment groups with 10 birds assigned to each of 64 floor pens (8 replicates/treatment) containing fresh pine shavings. The chicks were kept on a continuous lighting program and provided free access to feed and water throughout the trial. Individual body weights and feed consumption were recorded at 21 d of age and feed efficiency was calculated and corrected for mortality on a "bird-day" basis. Three birds per pen were then randomly selected for the collection of both tibiae, for a total of 48 tibiae (right and left) per treatment (8 replicates/treatment). Tibia samples were stored in plastic bags and frozen at -20°C until analyzed for breaking strength. Each tibia was brought to room temperature prior to assessing bone strength via a TA-HDi texture analyzer³, as described by Shaw *et al.* (2010). A fulcrum with a width of 3.0 cm supported each tibia and a round-based probe attached to a 50 kg load cell, with a crosshead speed of 1 mm/s, was used to break each bone. Experimentation was carried out in the facilities of the Auburn University Poultry Science Research Unit and animal handling procedures were in accordance with Auburn University's Institutional Animal Care and Use Committee (IACUC).

Treatments

The corn-soybean meal control diets (Table 1) included marginal (0.35%, 0.87%) or standard (0.45%, 1.0%) levels of npP and Ca. All other nutrients within the diets were formulated to meet or exceed all nutrient requirements set forth by the NRC (1994). An additional two

Table 1. **Ingredient and calculated analysis of broiler diets containing two levels of non-phytate phosphorus/calcium combinations, fed from 0–21 d of age**

Ingredients	Marginal Standard	
	(%)	
Ground yellow corn (7.5% CP)	55.71	54.67
Soybean meal (48% CP)	36.49	36.66
Poultry fat	3.65	4.03
Dicalcium phosphate (21.5% P; 18.5% Ca)	1.17	1.72
Ground limestone (38% Ca)	1.36	1.30
Sodium chloride	0.48	0.48
Trace-mineral premix ¹	0.25	0.25
Vitamin premix ²	0.50	0.50
L-lysine (98.5%)	0.12	0.12
DL-methionine (99.9%)	0.27	0.27
Total	100.00	100.00
Calculated Analysis (%)		
Metabolizable energy, kcal/kg	3086.00	3086.00
Crude protein	22.00	22.00
Calcium	0.87	1.00
Non-phytate phosphorus	0.35	0.45
Sodium	0.21	0.21
Lysine	1.35	1.35
Methionine	0.63	0.63
Methionine + Cystine	0.97	0.97

¹Supplied the following per kg of complete feed: 125 mg manganese; 1 mg iodine; 55 mg iron; 6 mg copper; 55 mg, zinc; 0.3 mg selenium.

²Supplied the following per kg of complete feed: 8,000 IU vitamin A (retinyl palmitate); 2,000 IU cholecalciferol; 8 IU vitamin E (di-tocopheryl acetate); 2 mg menadione; 5.5 mg riboflavin; 13 mg pantothenic acid; 36 mg niacin; 500 mg choline; 0.02 mg vitamin B₁₂; 5 mg folic acid; 1 mg thiamin; 2.2 mg pyridoxine; 0.05 mg biotin; 125 mg ethoxyquin.

diets were produced to include either 500 FTU of Phytase A or 500 FTU of Phytase B into the marginal diet. All experimental diets were pelleted at 79°C , crumbled, and provided from 0 to 21 d of age.

Phytase Enzymes

Both phytases utilized in this experiment were a 6-phytase and were originally derived from *Escherichia coli* and mass-produced into *Pichia pastoris* and *Schizosaccharomyces pombe*, respectively. The pH for the optimal activity of each enzyme, according to their respective manufacturer, is 2.5–3.5 and 4.5–5.0 for Phytase A⁴ and B⁵, respectively.

Statistical Analysis

Statistical analyses were performed on all data using JMP software (SAS, 2005). Data were arranged in a 2×4 factorial arrangement and were subjected to a one-way analysis of variance and means were separated by Tukey's Honestly Significant Difference procedure at a probability level of 0.05. Analyses of percentages involving mortality were performed following transformation using arcsine of

¹Strain 1: Cobb 700; Cobb-Vantress, Inc., Siloam Springs, AR

²Strain 2: Ross 708; Aviagen, Inc., Huntsville, AL

³Texture Technologies, Scarsdale, NY

⁴Optiphos; JBS United, Sheridan, IN

⁵Phyzyme XP; Danisco, Denmark

their square root.

Results

Initial chick weight differed ($P < 0.001$) between the two genetic strains, where chicks from Strain 1 were on average 1.7 g lighter than Strain 2 chicks at time of placement (data not shown). By 21 d of age, body weights did not differ ($P > 0.05$) between the two strains.

Regardless of genetic strain, at 21 d, the average body weight of birds fed the marginal npP-Ca diet with either Phytase A or B were heavier ($P < 0.05$) than that of birds fed the marginal npP-Ca diet with no enzyme, while average body weight of birds fed the standard diet was intermediate (Table 2). Total body weight gain from 0–21 d was greater (68 g or more; $P < 0.05$) for both marginal + enzyme diets as well as the standard npP-Ca diet than that of the unsupplemented marginal diet. Feed consumption was also significantly decreased ($P < 0.01$) for birds on the unsupplemented marginal diet in comparison with the standard and marginal + Phytase B diets. Despite the differences in body weight gain and feed consumption, no differences ($P > 0.05$) were detected among the dietary treatments for feed: gain. Bone breaking strength was decreased ($P < 0.001$) for birds fed the non-supplemented, marginal npP-Ca diet in comparison with the remaining three dietary treatments. No differences were detected ($P > 0.05$) for bone strength due to genetic strain or enzyme type. There were also no interactive effects between chick

strain and diet for any of the main effects.

Discussion

The results indicate a 7% decrease in body weight gain between the marginal and adequate npP-Ca diets, which was expected due to the known negative implications on weight gain associated with lowering P levels. This difference in body weight gain was likely due to the reduction in feed consumed by birds fed the marginal diet.

Supplementation of either Phytase A or B to the marginal diet significantly increased feed consumption to the normal level. This increase led to more than a 60 g improvement in body weight in comparison with the unsupplemented marginal diet. In addition, supplementation with either phytase enzyme improved bone strength, suggesting that each was capable of releasing the sufficient amount of npP from phytate P. These findings suggest that npP must be included into the broiler diet near levels recommended by the NRC (1994) or, if included at marginal levels, should be supplemented with a phytase enzyme to avoid reductions in performance and skeletal strength.

The two broiler strains performed equally well in terms of feed and growth performance. Previous research has yielded similar results with regard to live performance beyond two weeks of age, where differences were not detected between commercial broiler strains if all other parameters remained constant (Smith and Pesti, 1998;

Table 2. Live performance and bone breaking strength for two broiler strains fed diets with either 0.45 or 0.35% non-phytate phosphorus (npP), of which the latter may have contained a phytase source¹, from 0–21 days of age^{1,2}

	Day 21		Day 0–21		
	Weight (g)	Force (kg)	BWG (g)	FC (g)	F:G (g/g)
npP-Ca level					
Standard	783 ^{ab}	16.0 ^a	736 ^{ab}	1068 ^a	1.46
Marginal	733 ^b	11.5 ^b	685 ^c	984 ^b	1.43
+ Enzyme A ³	797 ^a	16.6 ^a	750 ^a	1052 ^{ab}	1.41
+ Enzyme B	795 ^a	16.0 ^a	748 ^{ab}	1102 ^a	1.48
SEM ⁴	17.0	0.70	16.4	22.4	0.028
Broiler strain					
Strain 1	774	14.8	728	1047	1.44
Strain 2	780	15.2	732	1055	1.45
SEM	13.0	0.53	13.0	15.2	0.023
Significance					
npP-Ca level	*	***	*	**	NS
Broiler strain	NS	NS	NS	NS	NS

¹ All values represent contrasts involving 64 pens, each with 10 chicks at start of experimentation.

² At hatch, Strain 1 had an average body weight of 46.5 g and Strain 2 had an average body weight of 48.2 g.

³ Each enzyme was included into the diet at 500 FTU/kg.

⁴ SEM: Pooled standard error of the mean.

^{a,b} Means with different superscripts in a column differ.

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

Applegate *et al.*, 2003; Wolanski *et al.*, 2006). While Yalcin *et al.* (2001) did not provide data on live performance, results on bone strength beyond two weeks of age for multiple commercial broiler strains was not found to differ, similar to that of the current study.

There were no statistical differences for live performance between the supplementation of Phytase A or B, however Phytase A resulted in a lower feed consumption (50 g less/bird than Phytase B). This resulted in the numerically lowest FCR among all groups. The literature has previously shown that phytase not only improve the digestibility of minerals such as Ca and P, but also proteins (Kornegay *et al.*, 1996; Yi *et al.*, 1996; Selle *et al.*, 2000), amino acids (Kornegay *et al.*, 1996; Yi *et al.*, 1996; Ravindran *et al.*, 2000), and starches (Ravindran *et al.*, 1999) digestibilities.

Overall, neither broiler strain or phytase source was found to have an effect on bone breaking strength. Regardless of the specific source, supplementation of the marginal npP-Ca diets with phytase was found to improve live and bone performance. Therefore, decreasing dietary levels of npP, while maintaining a 2:1 Ca to npP ratio, requires phytase supplementation to obtain normal growth performance and skeletal growth, regardless of commercial broiler strain.

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