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Research Article
Effect of the Phytase Inclusion in Broiler Breeder Diets on Fecal and Egg Characteristics

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Abstract

Objective: A study was conducted to determine the effect of phytase inclusion in broiler breeder diets on fecal and egg characteristics of individually caged females. **Materials and Methods:** A total of 184 female broiler breeders were fed growing and laying diets containing 0.7% or 2.7% calcium (Ca) and 0.35% or 0.12% available phosphorus (AvP), respectively, with one of four graded levels of phytase 0 (Control), 300, 600 and 1200 FTU kg^{-1} with 46 replicate cages/treatment at photostimulation. **Results:** Feeding 1200 FTU kg^{-1} of phytase produced greater fecal liquid portion at 31 week ($p \leq 0.001$) and 38 week ($p \leq 0.01$) while fecal phosphorus (P) was increased ($p \leq 0.01$) at 38 week in birds that had consumed either 600 or 1200 FTU kg^{-1} of phytase. There was no significant effect of phytase on egg characteristics or egg production. **Conclusion:** When formulating broiler breeder diets with phytase, attention should be made to the quantity of enzyme used to avoid increased liquid feces.

Key words: Broiler breeders, phytase, fecal moisture, liquid portion, fecal minerals

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Most poultry feed ingredients have historically been of plant origin, with the anti-nutritional factor phytate present as mixed salts of phytic acid[1]. Feedstuffs such as corn and soybean meal have poor bioavailability of phosphorus (P)[2]due to phytate. Furthermore, poultry lack the capacity to produce sufficient endogenous phytase to optimally utilize organic P present in feedstuffs[3]. Therefore, addition of highly bioavailable inorganic P sources has been used in poultry diets in order to meet P requirements. As a consequence, part of the dietary P not utilized by animals has been excreted, which has caused environmental concerns[4]. Strategies to economically reduce this environmental impact have included genetic selection of animals for reduced P requirement[5], reduced P safety margins during feed formulation and inclusion of phytase enzyme in feed to improve availability of phytate P[6,7]. All of these strategies have resulted in reduced inorganic P addition to diets and a more efficient use of P contained in feedstuffs. However, it has been suggested that addition of phytase has also led to increased availability of other minerals that were chelated to phytic acid such as calcium (Ca)[7-11]. Consequently, the effective Ca to available P ratio (Ca:AvP) may have been altered[7,12].

Plumstead *et al.*[13] reported that 300 FTU kg^{-1} dietary phytase reduced fecal moisture (FM) produced by floor-reared broiler breeder pullets when included in diets with 0.85% Ca and either 0.35 or 0.45% AvP. However, when these same birds were transferred to a two-thirds slat laying facility exhibited numerically increased FM when 500 FTU kg^{-1} dietary phytase was included in laying diets including 2.7% Ca and either 0.22 or 0.45% AvP. This difference in FM was suggested to have been due to phytase dosage, dietary Ca level, or the relative absence of P recycling on slats versus floor[14]. Nonetheless, these data suggested an increased FM due to phytase independent of dietary AvP in broiler breeder laying diets. Researchers have also reported that an inappropriate Ca:AvP ratio could negatively affect water intake[15] and water retention[16,17], which could result in an increased FM[18]. These facts suggested that mineral content and/or phytase activity in feed had to be appropriately manipulated in order to avoid wet feces that could have detrimental effects on animal welfare[19] as well as contamination of broiler hatching eggs. The objective of the present study was to determine the effect of inclusion of phytase in broiler breeder diets on FM, fecal liquid portion (LP), fecal Ca and P, egg quality and egg production.

MATERIALS AND METHODS

General: Care of the birds in this study conformed to the Guide for Care and Use of Agricultural Animals in Research and Teaching[20]. Broiler breeders were reared in a fan-ventilated enclosed house with litter floors on an 8 h photoperiod to 21 week of age using standard management practices. The pullets were then placed individually into 0.46 m×0.33 m×0.41 m (length×width×height) cages at 21 week of age and fed with individual polyvinyl chloride (PVC) feeders that minimized cross-contamination between cages. Day length was increased to 14 h at 21 week, 15 h at 23 week (10 d after housing), 15.5 h at 5% lay (25 week) and 16 h at 50% production, which was maintained for the duration of each experiment. House temperature was maintained between 16 and 27°C using curtains and/or heaters and circulating fans. Drinking water was analyzed for mineral content at the NCDA&CS Agronomic Division Plant/Waste/Solution Section Laboratory with levels of Ca, P, potassium, sodium, chlorine and magnesium found to be 15.10, 0.29, 3.93, 8.62, 10.30 and 3.85 ppm, respectively. Individual cages were fitted with inclined aluminum pans to collect feces and a 250 mL beaker to collect the liquid portion (LP) that drained from feces daily (Fig. 1). The aluminum fecal collection pans were located with enough distance from the drinker and feeder of each cage to avoid water and feed contamination of the feces. There was an empty cage between birds to further guard against cross-contamination.

Fig. 1: Aluminum feces collecting tray with hanging beaker to collect liquid portion (LP) of the feces. The slightly inclined trays were placed with sufficient distance from the drinkers and feeders and an empty cage between birds was left to minimize diet and sample cross-contamination

Table 1: Broiler breeder grower diets fed from 7-27 weeks of age

	Dietary treatments (FTU kg^{-1})			
	0	300	600	1200
Ingredients (%)				
Corn	64.63	64.63	64.63	64.63
Soybean meal (48% CP)	13.35	13.35	13.35	13.35
Wheat bran	16.60	16.60	16.60	16.60
Corn gluten meal	1.00	1.00	1.00	1.00
Poultry fat	1.00	1.00	1.00	1.00
Limestone	0.90	0.90	0.90	0.90
Dicalcium phosphate (18.5% P)	1.21	1.21	1.21	1.21
Salt	0.50	0.50	0.50	0.50
DL-methionine	0.09	0.09	0.09	0.09
L-lysine	0.07	0.07	0.07	0.07
Choline chloride (60%)	0.20	0.20	0.20	0.20
Vitamin premix[1]	0.10	0.10	0.10	0.10
Mineral premix[2]	0.10	0.10	0.10	0.10
Coccidiostat	0.05	0.05	0.05	0.05
Selenium premix[3]	0.10	0.10	0.10	0.10
Phytase[4]	0.00	0.017	0.033	0.066
Vermiculite (inert filler)	0.10	0.083	0.067	0.034
Calculated basal nutrient content[5]				
Metabolizable energy (kcal g^{-1})	2.80			
Crude protein (%)	14.13			
Calcium (%)	0.70			
Available phosphorus (%)	0.35			
Lysine (%)	0.70			
Methionine (%)	0.34			
Threonine (%)	0.49			
Methionine+cysteine (%)	0.57			
Sodium (%)	0.20			

[1]Vitamin premix supplied the following per kg of diet: 13,228 IU vitamin A, 3,968 IU vitamin D3, 66 IU vitamin E, 0.04 mg vitamin B12, 0.26 mg biotin, 3.96 mg menadione (K_3), 3.96 mg thiamine, 13.2 mg riboflavin, 22 mg d-pantothenic acid, 7.94 mg vitamin B6, 110 mg niacin and 2.2 mg folic acid. [2]Mineral premix supplied the following/kg of diet: Manganese 60 mg, zinc 60 mg, iron 40 mg, copper 5 mg, iodine 1.25 mg and cobalt 0.5 mg. [3]Selenium premix provided 0.2 mg Se kg^{-1} of diet (as Na_2SeO_3). [4]Phytase enzyme added at 0, 300, 600 and 1200 FTU kg^{-1}. [5]Formulations were based upon the control without added enzyme. Each diet was amended with phytase as a replacement for inert filler in this study. Calculated nutrient values shown above only reflect basal values and not any potential values added by phytase

The LP volume was measured separately from the remaining fecal material with a graduated cylinder on a daily basis. The feces and LP were then homogenized in an identified individual plastic bag. A sub-sample of mixed feces was placed in a collection pan and dried in an oven for 24 h at 95°C for determination of dry matter using AOAC Method 934.01[21] and FM. Samples of dried feces were ground (<2 mm) and subjected to mineral analysis as above. The remaining mixed feces were frozen for further analysis. A total of 184 Ross 708 strain broiler breeder females were fed 1 of 4 grower diets (Table 1) from 7-27 weeks of age before being fed 1 of 4 layer diets thereafter (Table 2). The 4 dietary treatments of each grower and breeder layer diet were created by amending basal diets with 0, 300, 600, or 1200 FTU kg^{-1} of a 6-Phytase derived from *Buttiauxella* sp. expressed in *Trichoderma reesei*. Diet formulations were based upon the control (0 FTU kg^{-1}) to which the various levels of phytase were added. This was an "on top" application of phytase as has been frequently employed in numerous commercial situations globally for a number of years (personal observations of the authors). The first-laid egg from each hen was used to measure weights of egg, yolk, shell and shell thickness. The LP and FM of each hen as well as fecal Ca and P were measured at 31 and 38 weeks of age. Egg production was recorded from onset of lay to 38 weeks of age.

Statistical methods: A one-way ANOVA using a completely randomized design with 46 individually caged replicate birds for each of the 4 per dietary treatments was employed. The general linear model of SAS[22] was used to analyze variables and differences among means were partitioned by LSMEANS. Differences were considered to be statistically significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

Egg weight and egg quality characteristics were not affected by dietary treatment (Table 3). Egg production was also not affected (data not shown for brevity). This suggested

Table 2: Broiler breeder layer diets fed from 28-43 weeks of age

	Dietary treatments (FTU kg^{-1})			
	0	300	600	1200
Ingredients (%)				
Corn	65.55	65.55	65.55	65.55
Soybean meal (48%CP)	17.08	17.08	17.08	17.08
Wheat bran	6.04	6.04	6.04	6.04
Corn gluten meal	1.00	1.00	1.00	1.00
Poultry fat	1.00	1.00	1.00	1.00
Limestone	6.91	6.91	6.91	6.91
Dicalcium phosphate (18.5%P)	0.00	0.00	0.00	0.00
Salt	0.50	0.50	0.50	0.50
DL-methionine	0.11	0.11	0.11	0.11
L-lysine	0.07	0.07	0.07	0.07
L-Threonine	0.03	0.03	0.03	0.03
Choline chloride (60%)	0.20	0.20	0.20	0.20
Vitamin premix[1]	0.10	0.10	0.10	0.10
Mineral premix[2]	0.10	0.10	0.10	0.10
Coccidiostat	0.05	0.05	0.05	0.05
Selenium premix[3]	0.10	0.10	0.10	0.10
Phytase[4]	0.00	0.017	0.033	0.066
Filler (Vermiculite)	0.15	0.133	0.117	0.084
Calculated basal nutrient content[5]				
Metabolizable energy (kcal g^{-1})	2.80			
Crude protein (%)	14.80			
Calcium (%)	2.70			
Available phosphorus (%)	0.12			
Lysine (%)	0.75			
Threonine (%)	0.53			
Methionine+cysteine (%)	0.62			
Sodium (%)	0.20			
Analyzed nutrient content				
Crude protein (%)	17.41	15.86	17.01	16.93
Calcium (%)	2.43	2.74	2.49	2.77
Total phosphorus (%)	0.45	0.45	0.47	0.45

[1]Vitamin premix supplied the following/kg of diet: 13,228 IU vitamin A, 3,968 IU vitamin D3, 66 IU vitamin E, 0.04 mg vitamin B12, 0.26 mg biotin, 3.96 mg menadione (K_3), 3.96 mg thiamine, 13.2 mg riboflavin, 22 mg d-pantothenic acid, 7.94 mg vitamin B6, 110 mg niacin and 2.2 mg folic acid. [2]Mineral premix supplied the following/kg of diet: Manganese 60 mg, zinc 60 mg, iron 40 mg, copper 5 mg iodine 1.25 mg and cobalt 0.5 mg. [3]Selenium premix provided 0.2 mg Se kg^{-1} of diet (as Na_2SeO_3). [4]Phytase enzyme added at 300, 600 and 1200 FTU kg^{-1}. [5]Formulations were based upon the control without added enzyme. Phytase was added as a replacement for inert filler. Calculated nutrient values shown above only reflect basal values and not any potential values added by phytase

Table 3: Effect of dietary phytase fed from 28-43 weeks of age on absolute and relative egg components of first laid eggs of broiler breeders

	Phytase (FTU kg^{-1})[1]					
Egg components	0	300	600	1200	SEM[2]	p-value
Egg weight (g)	45.5	44.6	45.9	44.6	0.5	0.76
Yolk (g)	11.5	11.3	11.4	11.0	0.1	0.36
Shell (g)	3.7	3.6	3.7	3.7	0.1	0.85
Albumen (g)	30.2	29.7	30.7	29.9	0.4	0.84

[a,b]Means in a row that possess different superscripts differ significantly ($p \leq 0.05$). [1]The 4 dietary treatments of each grower and breeder layer diet were created by amending basal diets with 0, 300, 600, or 1200 FTU kg^{-1} of an *Escherichia coli*-derived phytase enzyme (Danisco Animal Nutrition, Marlborough, UK). Diet formulations were based upon the control (0 FTU kg^{-1}) to which the various levels of phytase were added without reformulation. [2]Standard error of the mean (SEM) for 10 hens at first-laid egg

that all diets were more or less adequate. A reasonably wide range of inclusion of Ca, AvP and phytase would not be expected to have noticeable effects on these commonly measured variables within the flock ages studied. Thus, there was no evidence of any effects of the phytase enzyme on the nutritional value of the feed consumed by feed-restricted broiler breeder females.

Table 4 shows the effects of dietary phytase on fecal LP, FM, Ca and P at 31 and 38 weeks of age. Previous research has shown that an altered Ca:AvP ratio such as might be the case during the transition from rearing phase to laying phase could result in wet litter especially when phytase was used[23]. However, these data were taken several weeks after the onset of lay so this effect should not have been evident. Previous

Table 4: Effect of dietary phytase fed from 28-43 weeks of age on broiler breeder fecal liquid portion (LP) volume, fecal moisture, fecal calcium and fecal phosphorus content at 31 and 38 weeks of age

	Phytase (FTU kg^{-1})[1]					
Fecal variables	0	300	600	1200	SEM[2]	p-value
Liquid portion (mL)						
31 week	11.3^{b}	9.5^{b}	7.9^{b}	43.0^{a}	3.8	0.01
38 week	22.8^{b}	19.9^{b}	23.8^{b}	47.5^{a}	3.6	0.01
Fecal moisture (g/100 g)						
31 week	77.6	75.8	75.9	78.4	1.2	0.34
38 week	84.0	79.2	81.5	79.9	1.6	0.17
Calcium (mg kg^{-1} DM[3])						
31 week	71,990	61,200	55,210	71,630	5759	0.70
38 week	76,586	83,200	61,357	69,500	7682	0.79
Phosphorus (mg kg^{-1} DM[3])						
31 week	7,725	8,956	9,233	7,225	351	0.13
38 week	6,457^{b}	5,493^{b}	11,704^{a}	10,904^{a}	817	0.01

^{a,b}Means in a row that possess different superscripts differ significantly ($p \leq 0.01$). [1]The 4 dietary treatments of each grower and breeder layer diet were created by amending basal diets with 0, 300, 600, or 1200 FTU kg^{-1} of an *Escherichia coli*-derived phytase enzyme (Danisco Animal Nutrition, Marlborough, UK). Diet formulations were based upon the control (0 FTU kg^{-1}) to which the various levels of phytase were added without reformulation. [2] Standard error of the mean (SEM) for 10 hens at 31 and 38 week of age. [3] %DM of fecal samples were used to calculate nutrient contents on dry matter basis

research in commercial layers has also demonstrated that increased dietary Ca could cause a temporary increase in water intake and FM[15]. Smith *et al.*[24] concluded that an increased dietary AvP such as would be expected with the use of phytase led to a significant linear increase in water intake by commercial layers and consequently to an increased FM. However, effects on FM were not evident in the present study. Thus, differences in water intake due to Ca, AvP, or phytase probably did not occur. However, the 600 and 1200 FTU kg^{-1} diets did increase ($p \leq 0.01$) P excretion compared to the other treatments at 38 week of age (Table 4). This was interpreted to mean that 300 FTU kg^{-1} of phytase possessed insufficient enzyme activity to fully disrupt the phytate molecule as compared to the greater dosages. It has been previously proposed that destruction of the phytate molecule would release P as well as a number of chelated cations[25-27] such as Ca. However, this was not evident in the fecal Ca data of the present study. This may help explain why there were no enzyme effects on egg quality or egg production.

Interestingly, a clear relationship between LP volume and FM was not evident, since greater LP volume was not associated with greater FM (Table 4). The LP volume was increased at both 31 week ($p \leq 0.001$) and 38 week of age ($p \leq 0.01$) by feeding 1200 FTU kg^{-1} phytase. This was probably due to an increased digestion of the phytate molecule as FM did not differ (Table 4). An increased LP may be explained by altered water-holding capacity of the feces. Kalmar *et al.*[28] observed that African gray parrots fed fine versus coarse particle size feed produced similar FM (~72%) but excreta consistency was significantly different. This effect was attributed to the differential liberation and digestion, of non-starch polysaccharides (NSPs) due to different feed particle sizes (i.e. surface area) that altered the water-holding capacity of the feces. The greatest dosage of phytase in the present study probably degraded hygroscopic[29,30] organic macromolecules in the feces such as starch and proteins[31] such that water could no longer be held within the feces. This probably caused increased LP. There appeared to be a threshold effect on LP in the present data that was independent of FM. The effect on LP occurred somewhere above 600 FTU kg^{-1} in the present study. Plumstead *et al.*[13] and Harms *et al.*[14] also reported somewhat differential effects of phytase in broiler breeder diets in that 300 FTU kg^{-1} in a growing diet reduced FM while 500 FTU kg^{-1} increased FM in a laying diet. The increased LP observed in the present research could have a potential negative influence on hatching egg sanitation and broiler chick quality. A better understanding of *in vivo* phytase activity in feed-restricted broiler breeder hens is needed in order to safely use phytase at a dosage that allows for optimum utilization of nutrients without increasing LP. It will be important to remember that various hygroscopic properties of feces could be degraded during phytase action on its substrate, dependent upon dosage.

CONCLUSION AND FUTURE RECOMMENDATIONS

It was concluded that, under the conditions of this experiment, inclusion of phytase at 1200 FTU kg^{-1} to a broiler breeder layer diet can increase the liquid portion (LP) of the feces. Further research needs to investigate more specifically the conditions under which high dosages of

phytase can affect liquid feces in broiler breeders as an increased LP will pose a sanitation issue for broiler hatching eggs.

SIGNIFICANCE STATEMENT

This study discovered that adding phytase at a high dose increased liquid feces production in broiler breeders. This is a critical aspect in broiler breeder production as it affects hatching egg sanitation and quality of chicks going into the human food chain. This study will help researchers and poultry producers incorporate phytase correctly in their feed formulation.

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