Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2020 / v.22 / n.1 / 001-006

http://dx.doi.org/10.1590/1806-9061-2018-0890

Original Article

Effects of Phytase Inclusion in Broiler Breeder Diets During Early Lay on their Fecal and Egg Characteristics

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■Keywords

Broiler breeders, fecal minerals, fecal moisture, liquid portion, phytase.

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Submitted: 25/March/2019
Approved: 23/December/2019

ABSTRACT

This study investigated the effects of phytase inclusion in broiler breeder diets on fecal and egg characteristics. A total of 48 female broiler breeders were evaluated in this study from 21 to 31 weeks of age. The dietary treatments were fed from 30 to 31 wks of age, and included a Positive Control (PosCon) diet, containing 3.0% calcium and 0.50% available phosphorus (AvP); a Negative Control (NegCon) diet, with 3.0% calcium and 0.25% AvP; Negative Control diet + 275 FTU/kg phytase (NegCon+275), and Negative control diet + 550 FTU/kg phytase (NegCon+550). Egg, yolk, albumin, and eggshell weight, albumin height, and eggshell thickness were measured. Fecal parameters included fecal moisture, liquid portion, and mineral content. After 14 d on the experimental diets during the onset of lay, the NegCon+550 diet increased ($p<0.01$) fecal moisture content. In general, hens fed the highest enzyme level (NegCon+550) excreted fewer ($p<0.05$) divalent and trivalent cations, which included Al, Fe, Mg, Mn, and Zn. Fecal Na and K levels were not affected by dietary treatments. The NegCon+550 diet increased fecal P when compared with the NegCon and the NegCon+275 diets. The NegCon+550 and PosCon diets exhibited similar fecal P. No significant effects on egg characteristics were observed. It was concluded that during early lay, various signs of fecal changes would probably be observed at phytase dosages above approximately 500 FTU/kg characterized by increased fecal moisture content and excretion of P in broiler breeders.

INTRODUCTION

It has been long established that plant feed stuffs contain phytate, which is considered an anti-nutritional factor for poultry (Anderson, 1912), as they do not produce sufficient endogenous phytase to fully utilize the phosphorus (P) trapped as phytic acid (Maenz & Classen, 1998). Therefore, P bioavailability in common feedstuffs, such as corn and soybean meal, is limited (NRC, 1994). Consequently, the addition of highly-bioavailable inorganic phosphorus (P) sources to poultry diets has become a common practice. However, excessive dietary P, which was not utilized by the body, is excreted in the feces causing environmental pollution (Foy & Withers, 1995). Several strategies have been employed to reduce this environmental impact. The PosCon birds (Ca:AvP ratio ~6) exhibited an intermediate P excretion, followed by the NegCon+550 hens. Such as genetic selection for reduced P requirement (Punna & Roland, 1999), reduced P safety margins in feed formulation, and the dietary inclusion of phytase enzymes to feed (Yi *et al.*, 1996; Selle & Ravindran, 2007). These strategies have effectively reduced inorganic P levels in poultry diets and the environmental consequences of poultry production.

Furthermore, the chelation capacity of phytic acid for minerals such as calcium (Ca), P, and magnesium has been reported to reduce the bioavailability of these minerals (Nolan *et al*., 1987; Karimi *et al*., 2013). Over the past two decades, phytase enzymes have been introduced to increase the availability of P, Ca, and zinc bound by the phytate molecule (Adeola *et al*., 2004; Dilger *et al*., 2004).

According to Plumstead *et al*. (2007), adding 300 FTU phytase/kg diet reduced fecal moisture (FM) of floor-reared broiler breeder pullets receiving diets with 0.85% Ca and either 0.35% or 0.45% AvP. However, during lay, FM increased when 500 FTU phytase/kg were included in diets with2.7% Ca and either 0.22% or 0.45% AvP. Differences in FM may be due to phytase inclusion level, dietary Ca offered, or the relative absence of P on slats versus floor rearing (Harms *et al*., 1984). It has also been reported that an inappropriate or imbalanced Ca to available P (Ca:AvP) ratio may negatively affect water intake (Leeson & Summers, 1987) and water retention (Guo *et al*., 2008; Enting *et al*., 2009). It has been suggested that dietary phytase changes effective Ca:AvP ratio (Selle & Ravindran, 2007; Naves *et al*., 2016), which may result in increased FM (Bedford *et al*., 2007). These data suggest that dietary mineral content and phytase activity in feed needs to be appropriately managed to prevent excessive FM, which may have detrimental effects on animal welfare (Francesch & Brufau, 2004) as well as contaminate eggs used to produce broiler chicks. Therefore, this trial aimed at determining the effect of the inclusion of phytase in broiler breeder diets during early lay on FM, mineral excretion, and egg quality.

MATERIALS AND METHODS

This experiment was designed and conducted in compliance with the Guide for Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010).

A total of 48 female Ross 708 (Aviagen, Huntsville, AL) broiler breeder pullets were individually placed at 21 wks of age in 0.33 m x 0.46 m x 0.41 m (w x l x h) cages. The experimental diets were provided in individual PVC feeders, allowing minimal cross-contamination between cages. A lighting program of 14 h of light in 21 wk, 15 h of light 10 d after housing, 15.5 h at 5% lay (25 wk), and 16 h from 50% rate of lay to the end of the experiment. House temperature was maintained between 16°C and 27°C using curtains, heaters, and circulating fans.

Individual aluminum pans were fitted to each cage in order to collect feces. A 250-mL beaker was attached to collect the liquid portion (LP) that drained from feces daily (Figure 1). This setup allowed for the separation of the LP and feces for purposes of measuring amount of liquid. The aluminum fecal collection pans were installed in a manner that avoided water and feed contamination of the feces. Additionally, an empty cage was left between birds to prevent any cross-contamination. A graduated cylinder was used to daily measure the LP separately from the fecal material. The feces and LP from each individual hen were then mixed and homogenized in duly-identified bag to ensure the analysis of total nutrients present in both LP and feces. A sub-sample was collected and dried in an oven at 95°C for 24 h for the determination of FM and dry matter content (AOAC, 2006). Dried feces were then ground, and a sample was analyzed for mineral content.

Figure 1 – Aluminum trays used for feces collection. Hanging beaker used to collect liquid portion (LP) of the feces. Trays are placed within sufficient distance from the drinkers and feeders, and an empty cage was left between birds to prevent sample and treatment cross-contamination.

A broiler breeder grower diet (0.9% Ca, 0.45% AvP) was fed from 21 to 28 wks of age, after which each birds was fed 154 g of feed/d of a standard broiler breeder layer diet (2.7% Ca, 0.37% AvP) for an additional 7 d before the experimental period began (Table 1). For the following 14 d (30 to 31 wks of age), the experimental diets were fed. A basal diet was formulated to contain 3.0% Ca, to which appropriate amounts of dicalcium phosphate, limestone, filler, and phytase were added. The dietary treatments (Table 2) included: positive control with 0.50% AvP (PosCon); negative control with 0.25% AvP (NegCon); NegCon with the addition of 275 FTU phytase/kg

Table 1 – Ingredient composition and calculated analysis of initial layer diet fed to 28-wk-old broiler breeders prior to the experimental period.

Ingredients	
	(%)
Corn	68.90
Soybean meal, 48% CP	19.50
Limestone	5.95
Wheat bran	0.06
Poultry fat	2.50
Dicalcium phosphate	1.79
Salt	0.50
Premixes[1]	0.68
L-Threonine	0.02
DL-Methionine	0.10
Calculated nutrients[2]	
Metabolizable energy (kcal/g)	2.90
Crude protein	14.50
Lysine	0.78
Methionine + Cysteine	0.62
Threonine	0.56
Calcium	2.70
Available phosphorus	0.37
Sodium	0.20

[1]Premixes provided the following (per kg of diet): vitamin A, 13,200 IU; vitamin D_3, 4,000 IU; vitamin E, 66 IU; vitamin B_{12}, 39.6 μg; riboflavin, 13.2 mg; niacin, 110 mg; D-pantothenate, 22 mg; menadione (K_3), 4 mg; D-biotin, 252 μg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; selenium (as Na_2SeO_3), 0.30 mg;; zinc, 120 mg; manganese, 120 mg iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1.0 mg; choline chloride, 1,200 mg; coccidiostat, 500 mg.

[2]The nutrient compositions were calculated from proximate analyses of all ingredients and the final diet composition was confirmed by proximate analyses.

Table 2 – Ingredient composition and calculated analysis of diets fed to 30 to 31-wk-old broiler breeders.

	Dietary Treatments[1]			
Ingredients	PosCon	NegCon	NegCon +275;	NegCon +550
	(%)			
Corn	67.04	67.04	67.04	67.04
Soybean meal (48% CP)	19.03	19.03	19.03	19.03
Limestone	6.28	7.18	7.18	7.18
Inert filler (Vermiculite)	2.59	2.39	2.38	2.36
Poultry fat	2.11	2.11	2.11	2.11
Dicalcium phosphate (18.5%P)	1.73	1.03	1.03	1.03
Salt	0.50	0.50	0.50	0.50
Premixes[2]	0.65	0.65	0.65	0.65
L-Threonine	0.04	0.04	0.04	0.04
DL-Methionine	0.03	0.03	0.03	0.03
Phytase[3]	0.00	0.00	0.014	0.028
Calculated nutrients[4]				
ME (kcal/g)	2.90	2.90		
Crude protein	14.20	14.20		
Lysine	0.75	0.75		
Methionine + Cysteine	0.63	0.63		
Threonine	0.56	0.56		
Calcium	3.00	3.00		
Available Phosphorus	0.50	0.25		
Sodium	0.20	0.20		

[1]The four dietary treatments consisted of PosCon = 0.50% AvP, NegCon = 0.25% AvP,NegCon+275 = NegCon plus 275 FTU/kg feed, and NegCon+550 = NegCon plus 550 FTU/kg feed. [2]Premixes provided the following (per kg of diet): vitamin A, 13,200 IU; vitamin D_3, 4,000 IU; vitamin E, 66 IU; vitamin B_{12}, 39.6 μg; riboflavin, 13.2 mg; niacin, 110 mg; D-pantothenate, 22 mg; menadione (K_3), 4 mg; thiamine, 4 mg; folic acid, 2.2 mg; pyridoxine, 8 mg; D-biotin, 252 μg; selenium (as Na_2SeO_3), 120 mg; zinc, 0.30 mg; manganese, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1.0 mg; choline chloride, 1,200 mg; coccidiostat, 500 mg. [3]Phytase was added to NegCon+275 and NegCon+550 diets at 275 and 550 FTU/kg, respectively. [4] The nutrient compositions were calculated from proximate analyses of all ingredients and the final diet composition was confirmed by proximate analyses.

(NegCon+275); and NegCon with the addition of 550 FTU phytase/kg (NegCon+550). Diets were offered in coarse mash form. The phytase enzyme product was an *Escherichia coli*-derived 6-phytase added "on top" of the basal diet without using a matrix value.

After feeding the 48 females the standard layer diet (Table 1) for 7 d, hens were classified in three blocks based on LP production (high, average, and low LP). Four hens per LP block were assigned to each of the four dietary treatments (Table 2). Fecal LP and FM were determined on d 0 (pretreatment) and d 14 feeding the four experimental diets. The change in percentage FM (Δ%FM) while consuming the 4 experimental diets was calculated as follows: Δ%FM = %FM@14 d - %FM@0 d. Positive values indicate wetter feces with respect to d 0 d FM base value. Egg weight, eggshell weight, eggshell thickness, yolk weight, and albumen weight and height were determinedin two eggs per hen at 31 wks of age.

A randomized block design with 12 replicate hens per dietary treatment and four replicate hens per LP block (low, average, and high LP producers) was employed. The general linear model of SAS (2011) was used to analyze the variables. The LSMEANS command of SAS was used to partition differences among the means. Statistical significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Egg weight and egg quality parameter results are presented in Table 3, and no influence of dietary treatments were detected $p>0.05$), suggesting that all diets contained adequate nutrient levels. No differences were observed due to the dietary treatments in the production of LP volume (Figure 2) during the entire experimental period. However, LP differences were verified during the first period (d 0-1), when dietary Ca level wasincreased to 3.0% and AvP content was altered. The PosCon group exhibited the greatest transient increase in LP while NegCon+550 exhibited the least change. Previous research in commercial layers demonstrated that increasing dietary Ca caused a temporary increase in water intake and FM (Leeson & Summers, 1987). Moreover, Smith *et al*. (2000) found that increasing dietary AvP levels in commercial layer diets caused a significant linear increase in water intake that consequently increased FM. Indeed, PosCon diet, containing the highest inorganic P level, resulted in the most pronounced transient increase in LP. There was considerable sensitivity to altered Ca:AvP ratios in these laying birds, which may affect the contamination of hatching eggs. The hens fed the NegCon+550diet, which contained the greatest amount of enzyme, exhibited greater FM (Figure 3) on d 14 when compared to the other three dietary treatments ($p<0.05$). Therefore, theLP and FM of the evaluated broiler breeder hens dynamically responded to altered Ca:AvP, either with or without phytase.

These results demonstrated that higher phytase inclusion levels resulted in greater enzymatic digestion of phytate, as expected. However, only the birds fed the diet with the highest enzyme inclusion (NegCon+550) exhibited greater fecal moisture (FM) on d 14 (Figure 3). The NegCon+550 hens exhibited approximately 10% greater FM when d 0 and d 14 were compared. Presumably, this was due to greater digestion of the phytate molecule. It is known that phytate has the capacity to chelate positively-charged cations, and form complexes with starch and proteins/ aminoacids (Humer *et al.*, 2015). As a consequence, phytase may release these potentially hygroscopic organic macromolecules (Hori *et al.,* 2001; Castro-Freitas, 2005; Selle *et al.,* 2000), contributing to increase FM.

Table 3 – Effect of inclusion of phytase in diets fed to 30 to 31-wk-old broiler breeders on egg quality variables measured at 31wks of age.

	Dietary Treatments[1]					
Variable	PosCon	NegCon	NegCon +275	NegCon +550	SEM[2]	*p*-value
Egg weight, g	54.66	54.80	56.75	55.91	1.10	NS
Yolk weight, g	15.72	15.94	15.67	15.53	0.44	NS
Albumen weight, g	33.52	33.59	35.67	35.06	0.76	NS
Albumen height, mm	7.81	8.40	9.04	7.93	0.30	NS
Shell Weight, g	5.42	5.28	5.42	5.32	0.13	NS
Shell Thickness, mm	0.40	0.39	0.39	0.39	<0.01	NS

[a,b]Means in a row that possess different superscripts significantly differ ($p \leq 0.05$).

[1]Thefour dietary treatments consisted ofPosCon = 0.50% AvP, NegCon = 0.25% AvP,NegCon+275 = NegCon plus 275 FTU/kg feed, and NegCon+550 = NegCon plus 550 FTU/ kg feed.

[2]Standard error of the mean (SEM) for n=12 hens per diet.

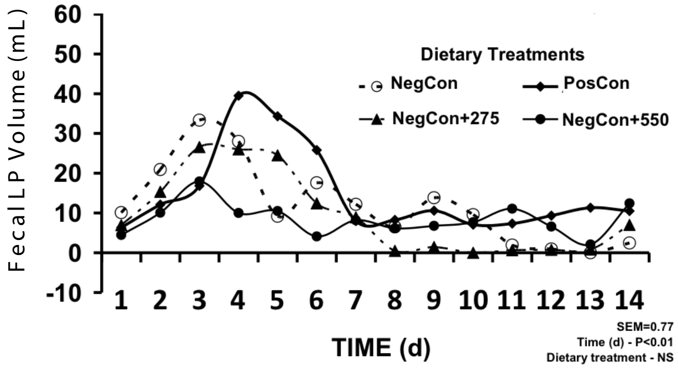

Figure 2 – Daily fecal liquid portion (LP) volume produced during the first 14 d on dietary treatments. The four dietary treatments consisted of PosCon = 0.50% AvP (diamond), NegCon = 0.25% AvP (circle), NegCon+275 = NegCon plus 275 FTU/kg feed (triangle), and NegCon+550 = NegCon plus 550 FTU/kg feed (dot). Standard error of the mean (SEM) for n=12 hens per diet. There was a significant effect of time ($p<0.01$), but no dietary treatment effect.

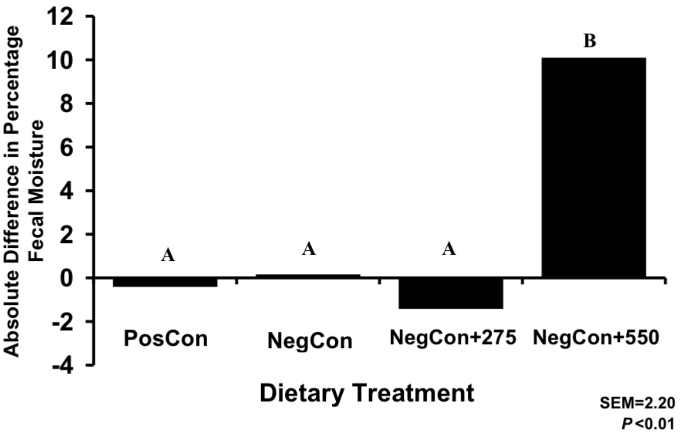

Figure 3 – Effect of inclusion of phytase in low available phosphorus broiler breeder diets on the absolute change in percentage fecal moisture (FM) after 14 d of dietary treatments. The four dietary treatments consisted of PosCon = 0.50% AvP, NegCon = 0.25% AvP, NegCon+275 = NegCon plus 275 FTU/kg feed, and NegCon+550 = NegCon plus 550 FTU/kg feed. Standard error of the mean (SEM) for n=12 hens per diet. [A,B]Means with different superscripts are significantly different at $p<0.01$.

A clear relationship between FM and LP volume was not evident in this study. Greater LP volume values did not result in greater FM. Other researchers have reported that this maybe explained by a difference in water-holding capacity of the feces. Previously, Kalmar *et al.* (2007) observed that African gray parrots fed fine or coarse particle-size pelleted feed produced similar FM (~72%). However, they also found that excreta texture was significantly different. They attributed their observations to the differential release of non-starch polysaccharides due to feed particle size (i.e., surface area) and microbial action upon them. It has been reported that these factors could influence fecal water-holding capacity (Francesch & Brufau, 2004).

Another factor that may have affected FM and LP was the fact that no Ca matrix value was assigned to the phytase. This resulted in slightly different Ca:AvP ratios among the treatments. Previous research showed that altered Ca:AvP ratio resulted in wet litter when phytase was used (Pos *et al.,* 2003). This could be mechanistically similar to the present data, but increased FM was only evident when the higher enzyme dosage was used.

Table 4 shows the effect of feed treatments on the fecal concentration of minerals on d 14. Birds fed the greatest amount of enzyme (NegCon+550 diet) excreted less divalent and trivalent cations and more P. Thus, a significant phytase effect was demonstrated at the greater dosage only. It has been previously proposed that the breakdown of the phytate molecule by phytase also releases chelated cations (Sebastian *et al*., 1998). The present data suggest that only the greatest dosage (NegCon+550) sufficiently disrupted the phytate molecule to release several trapped cations.

Table 4 – Effect of inclusion of phytase in diets fed to 30 to 31-wk-old broiler breeders on fecal mineral excretion at 31wks of age.

	Dietary Treatments[1]					
Mineral	PosCon	NegCon	NegCon+275	NegCon+550	SEM[2]	*p*-value
	(mg/kg DM)					
Calcium	64,907[B]	83,042[A]	82,160[A]	73,820[AB]	3974	<0.01
Phosphorus	17,355[ab]	13,342[b]	13,599[b]	21,048[a]	2128	0.05
Sodium	282	296	263	339	26	NS
Potassium	25,562	24,030	25,080	26,187	730	NS
Aluminum	6,550[A]	5,678[A]	5,785[A]	4,499[B]	296	<0.01
Iron	7,636[A]	6,131[BC]	6,916[AB]	5,452[C]	329	<0.01
Magnesium	13,781[A]	12,668[AB]	12,930[A]	10,985[B]	459	<0.01
Manganese	715[A]	680[AB]	625[B]	524[C]	29	<0.01
Zinc	512[a]	519[a]	478[ab]	437[b]	18	<0.05

[a,b]Means in a row that possess different superscripts are significantly different at $p<0.05$.

[A,B] Means in a row that possess different superscripts are significantly different at $p<0.01$.

[1]The four dietary treatments consisted of PosCon = 0.50% AvP, NegCon = 0.25% AvP,NegCon+275 = NegCon plus 275 FTU/kg feed, and NegCon+550 = NegCon plus 550 FTU/kg feed.

[2]Standard error of the mean (SEM) for n=12 hens per diet.

Furtermore, Table 4 clearly shows the resemblance in the response of NegCon and NegCon+275 birds with regards to fecal Ca and P content. This suggests that a low level of phytase activity or that a Ca:AvP ratio of around 12 caused increased Ca and reduced P excretion. It must be remembered that all diets, except for the positive control, had the same total P content. Thus, it could be suggested that the fecal P of PosCon was inorganic while that of NegCon+550 (greatest enzyme) was phytate-derived.

In conclusion, this study demonstrated the effects of adding stepwise doses of phytase on FM of broiler breeder hens during the early laying period. The use of the greater enzyme dose increased FM. A greater enzyme dosage or altered Ca:AvP ratio appeared to either increase water intake anddecrease the water-holding capacity of the feces. Thus, it was concluded that the addition of phytase at 550 FTU/kg to a broiler breeder layer diet increased FM during the onset of lay. Also, some ions and molecules with different electrochemical and hygroscopic properties were released only at the greatest phytase dosage. These data also demonstrated that broiler breeder hens required time to adapt to altered dietary Ca and AvP during early lay. This was observed irrespective of the presence or absence of dietary phytase. These results suggest that various signs of fecal changes may probably be observed at phytase dosages above approximately 500 FTU/kg.

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