MEDWELL PUBLISHING
Journal of Animal and Veterinary Advances 8 (6): 1096-1100, 2009
ISSN: 1680-5593

Effect of the Phytase Supplementation in Diets for Broilers Chickens on Growth and Phosphorus Absorption

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Abstract: Phosphorus (P) is a mineral required by all domestic animals to meet its requirements. Phytase is an enzyme that liberates phosphate by hydrolysis from phytate, to make it more available for absorption in gastrointestinal tract of not-ruminant, like birds and pigs. Experimental diets used with phosphorus and phytase were evaluated to estimate productive parameters of broiler chickens and simultaneously to evaluate as a test or absorption, with the phosphorus in the diets. In 1st trial, feed intake, daily gain and feed efficiency were measurement in 6 treatments diets included 3 phosphorus levels (0, 50 and 100%, of requirements) and 2 phytase (0 and 600 Units). Feed intake was increased by phosphorus ($p<0.005$) and phytase ($p<0.001$). Body weight gain was elevated by effect of phosphorus ($p<0.001$) and phytase ($p<0.001$) and their interaction ($p<0.001$). Phosphorus ($p<0.04$) and phytase ($p<0.03$) influenced on feed efficiency and an interaction was observed ($p<0.05$). In 2nd test, phosphorus absorption apparent, P intake was influenced by phosphorus level ($p<0.001$) and phytase ($p<0.001$). Phosphorus excretions also was increased by P level ($p<0.01$) and phytase contents ($p<0.001$) in grams per day. Phosphorus retention was influenced by phosphorus ($p<0.001$), phytase ($p<0.001$) and their interaction ($p<0.003$). Phosphorus content influenced ($p<0.043$) on percent tibias-bones ash and by phytase ($p<0.003$). In content by percent of tibias phosphorus was increased by P level ($p<0.036$), although not for phytase ($p>0.05$). Phytase aid to use phosphorus of broilers diets and makes efficient the feed intake and the phosphorus increase in bones.

Key words: Phytase, broilers, performance, phosphorus absorption, mineral, bones

INTRODUCTION

Phytates represents a class of complex compounds of occurrence in nature that they can influence significantly nutritional and functional states. Their presence has been known for 1 century, although its cycle is not totally well known and this represents an area of active investigation (Maga, 1982).

Due to phosphorus importance in development of skeleton as much quantity as availability of phosphorus in feedstuffs became critical for growth of animals. The values of bio-availability for several phosphates are necessary, to formulate diets to complete requirements for birds, avoiding excess of P in the feed, because that increase phosphorus excretion, enhanced environmental contamination (Potter *et al*., 1995).

Body weight gain and tibia ash has been a routine measurement in trials of bio-availability of P (Sullivan and Douglas, 1990). Fritz and Roberts (1969), Fritz *et al*. (1969), Yoshida and Hoshii (1977), Potchanakorn and Potter (1987) and Potter (1988) also used successively the tibia ash calculations, as a simple measure or approach to determine bio availability of phosphorus of the different phosphate sources.

Biological preparations containing phytases, have shown to increase hydrolysis of phytic phosphorus of feeds for broiler chickens (Nelson *et al*., 1968). Supplementation with microbial phytase (*Aspergillus niger*) improved the use of phytic P of feedstuffs (Lei *et al*., 1994; Adeola, 1995; Kemme *et al*., 1997; O'Quinn *et al*., 1997). The use of phytase in broiler and laying hens are interesting because to releasing the phosphorus of feedstuffs and for their relief in benefit of environment (Simons *et al*., 1990; Sohail and Roland, 1999; Yan *et al*., 2003).

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The purpose of this study, was to evaluate the effect of adding 2 phytase with 3 phosphorus levels on feeding diets to broiler chickens and evaluate performance. All treatments with these diets were recorded for feed intake, weight gain and feed efficiency.

Another test was made in same treatments to calculate the apparent availability of phosphorus when phytase was used. And then, the birds were killed and bone samples were collected, removed the fat and then dried. Thus, tibia-bones aliquots were processed for determining phosphorus content.

MATERIALS AND METHODS

Diets: Day old broiler chicks Ross (n = 306), were used in experiment for 21 days to investigate phytase supplementation, on behavior, tibia ash and P retention of chickens fed diets with and without phosphorus and different phytase levels added. Chickens were distributed in 6 treatments, with 3 replications each of 17 chicks, in a factorial design 3×2 (Three phosphorus levels by 2 phytase levels). The treatments were: Without P and without phytase; 50% P and without phytase; 100% of P and without phytase; without P and 600 phytase units; 50% P and 600 phytase units and 100% P and 600 phytase units. Content of the diet was 23.0% of crude protein, 3,200 kcal kg^{-1}, 1.0% of Ca, 0.45%, 1,500 UI kg^{-1} Vitamin A and 10 UI kg^{-1} of Vitamin E.

Feeding trials: The birds were vaccinated since new born. Cages and the installations have feeders and water facilities. The study lasted from the 1st-3rd weeks of age. The chicks were housed in floor pens with sawdust as bed. The feeds were offered at the 9:00 and 15:00 h, feed rejection being weighed daily in the morning, before offering the 1st daily ration. The rejected food was weighed and subtracted from offered feed, to calculate feed intake.

Diets were formulated to meet requirements for broiler chicks by NRC (1994). Feed were offered in floor feeders during the 1st week and during the 2nd and 3rd week, in hanging feeders. Fresh water was offered *ad libitum* in large drinking bottle troughs.

Initial body weight was registered at 1st day old and later the chickens were weighed weekly on 7, 14 and 21 days of age, at the same hour and before offering daily ration of feed. The chicks were weighed by each replicate of all treatments. Feed efficiency was calculated as feed intake, which was required to increase 1 kg of weight of chickens.

After growth test, another trial was made to determine apparent phosphorus availability of same diets. In this test, 54 broilers chickens of the same lot at 3-4 weeks of age were placed in cages. Nine chicks for each treatment were housed in cages (3 per cage), designed for total feces collections. Feces were collected daily, during 3 days and then frozen for later analysis. The dried feces and the mixture samples were milled in a mill (Thomas-Wiley, model 4, Thomas Scientific). These samples were weighed and placed in muffle for ash contents (AOAC, 1997).

At the end of the test, the chickens were sacrificed to collect bone tibia samples and then were degreased and dried. The scoured tibias-bones samples were macerated and milled. Following samples were processed (Dickman and Bray, 1940) to determine phosphorus contents in a spectrophotometer.

Statistical analysis: All data were analyzed according to General Linear Model (GLM) procedure of (SAS, 1997).

RESULTS AND DISCUSSION

Table 1 shows average data of feed intake, daily gain and feed efficiency of broiler chickens assigned to 6 treatments in diets included 3 phosphorus levels (0, 50 and 100%) and 2 of phytase (0 and 600 units of phytase).

Feed intake: An increase for effect on feed intake due to phosphorus ($p<0.004$) and phytase level ($p<0.001$) and an interaction, was observed during the 1st week (P x Phy; $p<0.028$). In 2nd week the feed intake, was also affected by for phosphorus level ($p<0.001$) and phytase ($p<0.001$) and an interaction ($p<0.021$). In 3rd week feed intake was better by effect of phosphorus level ($p<0.03$) and phytase level ($p<0.001$). Also, an effect of phosphorus ($p<0.005$) and phytase ($p<0.001$), over increment of feed intake in total period, was observed.

Body weight gain: In the Table 1 the data of Body Weight Gain (BWG) are also observed. For the 1st week averages of BWG, was increase for phosphorus ($p<0.007$) and phytase level in diet ($p<0.001$). Weight gain in 2nd week was affected to increase by phosphorus ($p<0.001$), phytase level ($p<0.001$) and interaction ($p<0.001$). In 3rd week, similar results were seen in broilers due to phosphorus ($p<0.001$) and phytase ($p<0.001$) and an effect for interaction ($p<0.008$); on BWG. In complete period, phosphorus ($p<0.001$) and phytase ($p<0.001$) affected gain and an interaction of them (P x Phy: $p<0.001$).

Feed efficiency: Feed Efficiency (FE) in broiler chicks during 1st week was affected by phosphorus ($p<0.07$) and phytase level ($p<0.01$). In 2nd week, the FE was improved

Table 1: Feed intake, daily weight gain and feed efficiency of broiler chicks fed various phosphorus and phytase levels

Diet/	Phosphorus			Phytase (U kg^{-1})			p-value	
Item	Low	Medium	High	0	600	SEM[a]	P	Phy
Feed intake (g)								
0-7[1]	22.60	22.10	23.8	21.60	24.00	0.17	0.004	0.001
8-14[2]	46.90	48.70	54.2	45.00	54.90	0.62	0.001	0.001
15-21	62.50	68.60	75.5	53.00	84.80	1.68	0.030	0.001
0-21	44.10	46.50	51.2	39.90	54.60	0.54	0.005	0.001
Daily weight gain (g)								
0-7	14.00	14.80	16.2	13.40	16.60	0.23	0.007	0.001
8-14[3]	16.30	19.10	23.1	15.20	23.80	0.34	0.001	0.001
15-21[4]	25.90	36.50	48.2	22.80	51.00	1.32	0.001	0.001
0-21[5]	18.70	23.50	29.2	17.10	30.40	0.49	0.001	0.001
Feed:gain (g:g)								
0-7	1.62	1.51	1.48	1.61	1.46	0.02	0.070	0.010
8-14[6]	3.35	2.62	2.35	3.24	2.31	0.05	0.001	0.001
15-21	5.17	2.04	1.56	4.18	1.67	0.71	0.120	0.100
0-21[7]	3.38	2.06	1.80	3.01	1.81	0.24	0.040	0.030

[a]SEM, Standard Error of the Mean. 1): Pho x Phy ($p<0.028$), 2): Pho x Phy ($p<0.021$), 3): Pho x Phy ($p<0.001$), 4): Pho x Phy ($p<0.008$), 5): Pho x Phy ($p<0.001$), 6): Pho x Phy ($p<0.001$) and 7): Pho x Phy ($p<0.05$)

Table 2: Apparent digestibility of phosphorus on broiler chicks with several phosphorus and phytase levels in diet

	Phosphorus			Phytase (U kg^{-1})			p-value	
Item	Low	Medium	High	0	600	SEM[a]	Phosphorus	Phytase
P intake								
g day^{-1}	0.227	0.243	0.310	0.143	0.377	0.003	0.001	0.001
P excretion								
g day[1]	0.237	0.233	0.196	0.272	0.196	0.005	0.010	0.001
mg g^{-1} DM	3.420	2.460	1.500	4.160	0.760	0.253	0.020	0.010
P retention								
g day^{-2}	0.048	0.069	0.153	-0.053	0.233	0.005	0.001	0.001
Intake (%)[3]	-21.010	-7.950	30.160	-63.710	64.510	3.460	0.001	0.002
P digestibility (%)	18.390	17.560	29.420	2.520	41.070	1.460	0.006	0.001

[a]SEM, Standard Error of the Mean, [1]Pho x Phy ($p<0.001$), [2]Pho x Phy ($p<0.003$), [3]Pho x Phy ($p<0.003$)

Table 3: Tibia ash and P contents of broiler chicks fed with several inorganic phosphorous and phytase levels

	Phosphorus			Phytase (U kg^{-1})			p-value	
Item	Low	Medium	High	0	600	SEM[1]	Phosphorus	Phytase
Tibia ash (%)	10.90	12.87	17.78	10.16	17.54	1.005	0.043	0.003
Tibia, P (%)	9.79	12.43	20.45	14.05	14.26	1.49	0.036	0.945

[1]SEM, Standard Error of the Mean

by phosphorus level ($p<0.001$) as phytase level ($p<0.001$) and their interaction ($p< 0.001$) in chicken fed with those diets. In 3rd week feed efficiency was not affected neither by phosphorus or phytase level ($p>0.05$). In complete period the FE showed an effect of phosphorus level ($p<0.04$) and phytase ($p<0.03$) and also was observed an interaction ($p<0.05$).

Apparent digestibility of phosphorus: In Table 2, main effects of phytase and phosphorus level can be observed in chickens by diet consumed in the 2nd experiment. It was observed that phosphorus intake was influenced by phosphorus level ($p<0.001$) and phytase ($p<0.001$) in feed rations. Excretion of phosphorus by chickens similarly was influenced by phosphorus ($p<0.01$) and phytase ($p<0.001$) offered in diet, when being evaluated in grams per day an interaction was also observed and those values were influenced by high level of phosphorus and phytase of diet ($p<0.001$).

In the same way, can observed that P retention (g day^{-1}), was influenced by phosphorus and phytase in diets ($p<0.001$; $p<0.001$, respectively), where an interaction was observed ($p<0.003$). On the other hand, phosphorus retention, measured as intake percent, was also influenced by phosphorus ($p<0.001$) and phytase ($p<0.002$) in rations. Interaction, also ($p<0.003$) given by highest levels of phosphorus inclusion at 100% of requirement and 600 units of phytase. Phosphorus digestibility was higher due to effect of phosphorus level ($p<0.006$) and phytase level in diet ($p<0.001$), was 29.4 and 41.1%, respectively.

Tibia bone ash and p content: Table 3 shows effect of phosphorus and phytase addition to chickens diets in absorption trial. Phosphorus influenced ($p<0.04$) on percent of tibias-bones ash and same effect was showed for phytase contents ($p<0.003$) in diet. In content by

phosphorus percent of tibias bone, was observed that was influenced by P level ($p<0.04$), but not by phytase ($p>0.05$) in diets.

Results of weight gain, feed intake and feed efficiency of chickens which were offered them rations with different phosphorus levels and 600 units of phytase kg^{-1}, showed effects due to phytase additions, being observed in the weight gain and feed efficiency with a linear effect. This results agree with data of weight gain of other studies where 500 UF were used (Huff *et al*., 1998), 600 UF (Sebastian *et al*., 1996a and b; Um and Paik, 1998a), 800 UF (Yi *et al*., 1996). But differ with the results found by Roberson *et al*. (1994).

In 2nd trial, apparent absorption of P of feed in broiler diet, was observed, in all period, an effect of the supplementary P, phytase and their interaction. Also, a linear effect was observed, although a quadratic effect was not presented in same period, this effect was observed in the days 1 and 3 of experiment, this event has not been reported in other types of studies on phytase addition in broiler diets.

For the P content and tibias-bone ash of chickens, phytase did not show any effect, therefore, effect was due to phosphorus supplementation and in this sense the best percentages in P content and ashes, was observed in diets of 50% of P and 600 units of phytase kg^{-1}. This data agree with data reported by Qian *et al*. (1997), where, broiler chickens were fed with levels of 300, 600 and 900 units kg^{-1} of feed. They also found that phytase increased the body weigh gain lineally and ashes contents of fingers and phosphorus retention.

CONCLUSION

The results of this study show that the phosphorus is used more efficiently when add phytase to growing chickens diets. The effect of feeding high phytase diets becomes most evident before and until 21 days of age and phosphorus is necessary to maintain the normal growth of tibia bones.

The phosphorus releasing is retained as structural part of bones and the phytase addition has an effect on phosphorus it that found in ration ingredients for chickens.

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