ILEAL PHOSPHORUS DIGESTIBILITY IN BROILER CHICKENS FED LOW CALCIUM AND PHOSPHORUS DIETS SUPPLEMENTED WITH PHYTASE AND CHOLECALCIFEROL

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ABSTRACT
A total of 125 1-day-old Arbor Acres broiler chickens fed broiler starter till day 20 were used to investigate the effect of low calcium and phosphorus diets supplemented with phytase and cholecalciferol. On day 21, the chicks were weighed and allotted to 5 treatments with 5 replicates of 5 birds each in a randomized complete block design. The treatments comprised a positive control (PC, T1) without cottonseed meal (CSM) and four CSM-based semi-purified diets, negative control (NC, T2), NC+vitamin D3-10,000 IU/Kg (T3), NC+phytase (T4) and NC+phytase+vitamin D3 (T5). Titanium dioxide (TiO2) (5 g/Kg) was included as marker. Between days 25 and 27 post-hatch, samples of fresh excreta were collected once daily from each cage. On day 28, the birds were asphyxiated using carbon (IV) oxide and the birds dissected to obtain digesta from the distal two-third of the ileum. All data were analysed using the GLM procedure in SAS. On day 28, the remaining birds were killed and digesta from the 2/3rd of the ileum collected. Digestible P was significantly ($P<0.05$) improved for birds on T5 when compared with the other four diets. Digestible P in T4 did not differ ($P>0.05$) from the birds on T1. Ileal digestible phosphorus (IDP) significantly ($P<0.05$) reduced with a corresponding increase in the percentage apparent phosphorus digestibility (APD) in T3, while T4 and T5 did not differ from T1 in the two parameters. It can be concluded that phytase and vitamin D3 supplementation on cottonseed meal improved digestible P in broiler chickens.
Keywords: Cottonseed meal, phytase, vitamin D_3, ileal phosphorus, broilers

INTRODUCTION

Supplementation of broiler diets with microbial phytase and vitamin D_3 has been shown to improve calcium, copper, phosphorus and zinc utilization, and consequently reduce the excretion of these minerals in the faeces (Asadi *et al.,* 2023). The level of Ca in the diet may also affect the utilization of phytic acid-P through the formation of insoluble calcium phytate and/or reduction of phytase activity (Singh *et al.,* 2003). Cholecalciferol (vitamin D_3) plays a role in Ca and P absorption, and therefore influences their utilization. Mohammed *et al.* (1991) reported that cholecalciferol supplementation of poultry diets increases phytic acid-P utilization. Cholecalciferol is derived from 7-dehydrocholesterol in the skin of animals. Ultraviolet light transforms these compounds into calcitrol also known as 1, 25-dihydroxycholecalciferol, the vitamin D metabolite generally considered the most active. Metabolites of cholecalciferol may or may not improve phytic acid-P utilization in poultry or pig's diet (Liem *et al.,* 2009). Calcium to phosphorus ratio and phosphorus content of diets also influence the inorganic P release from phytate by phytase (Poulsen *et al.,* 2010). Animal feed contains high amount of calcium hence calcium content in diet can have a large impact on phytate P utilization and phytase efficiency (Mohammed *et al.*, 1991). Increasing the Ca to total P ratio may have negative impact on phytase activity (Qian *et al.,* 1996). The authors (Qian *et al.,*1996) reported that reducing dietary Ca:P ratio from 2:1 to 1.2:1 increased phytase efficiency by approximately 16% and improved performance and digestibility in weaning piglets. Similarly, Liu *et al.* (2013) concluded that lowering Ca:P ratio from 1.5:1 to 1:1 improved performance and P utilization in pigs fed low P corn soya bean meal based diets supplemented with phytase. Cottonseed meal has a high phytate content of 70% (Ravindran, 1996), hence Inclusion of the appropriate amount of CSM will provide information on the amount of phosphorus digestible in broiler diets. Hence the objective of this study is to determine the effect of supplementing low P diets containing CSM with vit D_3 and phytase on ileal P digestibility in broiler chickens.

Materials and methods

One hundred and twenty five 1-day-old Abor Acre broiler chickens were fed starter diet till day 20. The birds were raised on a deep litter system for 20 days and later replicated in cages. Pans were placed beneath each cage for fresh excreta collection daily. Positive control (PC) diet with required Non Phytate Phosphorus (NPP) -. (4.84 g/kg diet) and a Negative control (NC) diet in which the NPP is reduced by 65% (i.e. from 4.84 to 1.69 g/kg diet for CSM) was formulated. Three other diets each for CSM in which the NC diet is supplemented with either 1000 FTU phytase/kg diet, 10,000 IU.Vit D_3/kg diet or both was formulated, making a total of 5 semi-purified diets. On day 21, the chicks were individually weighed and allocated to the 5 diets in a randomized complete block design with 5 replicates of 5 birds each. Five cages were randomly assigned to each of the 5 treatments. Titanium dioxide was included at the rate of 5g/kg diet as an indigestible marker. The broiler chickens were fed for 8 days and fresh excreta collected on day 5 to 7. On day 8, ileal digesta was harvested after asphyxiation with CO_2.The trial lasted 8days.

Table 1. Gross composition of experimental diets (g/kg)

	PC	NC	NC+Vit D_3	NC+Phy	NC+Phy+Vit D_3
Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Cottonseed meal	0.00	315.00	315.00	315.00	315.00
Cassava Starch	608.00	469.00	468.75	459.00	458.75
Wheat gluten	250.00	100.00	100.00	100.00	100.00
Soya oil	10.00	2.00	2.00	2.00	2.00
Dextrose	90.00	90.00	90.00	90.00	90.00
Methionine	1.00	1.00	1.00	1.00	1.00
Lysine	1.00	1.00	1.00	1.00	1.00
Limestone	17.00	12.00	12.00	12.00	12.00
Vitamin-Premix	2.50	2.50	2.50	2.50	2.50
Common salt	2.50	2.50	2.50	2.50	2.50
Phytase (FTU/kg)	0.00	0.00	0.00	10.00	10.00
Cholecalciferol (g/kg)	0.00	0.00	0.25	0.00	0.25
TiO_2 Premix	5.00	5.00	5.00	5.00	5.00
Dicalcium phosphate	13.00	0.00	0.00	0.00	0.00
Total	**1000.00**	**1000.00**	**1000.00**	**1000.00**	**1000.00**
Calculated Nutrients					
ME Kcal/Kg	3575.37	3158.31	3157.44	3575.37	3575.37
CP (g/kg)	218.00	217.61	217.61	217.61	217.61
Ca (g/kg)	10.85	5.64	5.64	5.64	5.64
Total P (g/Kg)	4.84	4.06	4.06	4.06	4.06
NonPhytateP (g/kg)	4.84	1.69	1.69	1.69	1.69
Phytate P (g/kg)	0.00	2.36	2.36	2.36	2.36
Ca : NPP ratio	2.24	3.33	3.33	3.33	3.33
Ca : P ratio	2.24	1.39	1.39	1.39	1.17

[1]*Composition of vitamin premix per kg of diet: vitamin A, 12500 I.U; vitamin E, 40mg; vitamin K_3, 2mg; vitamin B_1, 3mg; vitamin B_2, 5.5mg; niacin, 5.5mg; calcium pantothenate, 11.5mg; vitamin B_6, 5mg; vitamin B_{12}, 0.025mg;choline chloride, 500mg, folic acid,1mg; biotin, 0.08mg; manganese, 120mg;iron 100mg; zinc, 80mg; copper, 8.5mg; iodine, 1.5mg; cobalt, 0.3mg;selenium, 0.12mg, anti-oxidant, 120mg,* [2]*Phytase premix prepared by mixing phytase with maize.* [3]*Titanium dioxide premix prepared by mixing 1g of titanium dioxide with 4g of maize; NC:Negative Control, PC:Positive Control, Phy:Phytase, Vit D_3:Vitamin D_3,NNP:Non phytate phosphorus*

Chemical and Statistical Analysis

Samples of CSM, experimental diets, digesta and excreta were analysed for energy according to the methods of AOAC (2000). Titanium concentration was determined using a colorimetric assay (Short *et al*., 1996). Data were analysed using the GLM procedure of SAS (SAS Institute, 2012).

RESULTS AND DISCUSSION

Table 2 shows the results for Ileal, digested and apparent phosphorus digestibility and tibae bone ash of broilers fed cottonseed meal-based diets. Ileal phosphorus in the diet supplemented with vitamin D_3 (T3) significantly ($P<0.05$) reduced with a corresponding increase in the percentage apparent phosphorus digestibility (T3). Negative control diet supplemented with phytase (NC+Phy)-T4 and NC+Phy+vit D_3 (T5) did not differ ($P>0.05$) from the PC (T1) control at the ilea and in the apparent digestibility of the broilers. Digestible P was significantly ($P<0.05$) improved for birds on T5 when compared to the other four diets (T1, T2, T3 and T4). Digestible P in T4 did not differ ($P>0.05$) from the birds on the PC (T1) diet. Percentage tibiae bone ash in T3 did not differ ($P>0.03$) significantly from the PC diet, T4 and T5 but percentage tibiae bone of birds on T4 improved significantly ($P<0.05$) when compared with birds on the PC diets.

Table 2. Ileal, digested and apparent phosphorus digestibility of 28-day-old broilers fed cottonseed meal-based diets

	PC	NC	NC+VitD_3	NC+Phy	NC+Phy+VitD_3	SEM
Parameters	T1	T2	T3	T4	T5	
P (g/kg of diet)	0.87^{e}	1.06^{b}	0.88^{c}	0.87^{d}	1.11^{a}	0.21
Ileal P (g/KgDMI)	0.20^{ab}	0.26^{a}	0.05^{c}	0.08^{bc}	0.11^{bc}	0.25
Digested P(g/KgDMI)	0.67^{c}	0.80^{bc}	0.83^{b}	0.79^{bc}	0.95^{a}	0.29
APD (%)	76.61^{b}	75.81^{b}	94.61^{a}	90.64^{ab}	90.11^{ab}	2.53
Tibiae bone ash (%)	20.88^{b}	25.83^{a}	24.67^{ab}	27.31^{a}	21.15^{b}	0.73

^{abc} Means in a row with different superscripts are significantly different from each other ($P<0.05$)PC= Positive control, NC: Negative control, Phy: Phytase, Vit D_3: Vitamin D_3, P:Phosporus, APD: Apparent phosphorus diesibility

In this study, ileal P significantly reduced with the supplementation of vit D_3 and a corresponding increase in digestible P for birds fed a combination of vit D_3 and phytase (T5), percentage apparent P digestibility (APD) improved with the supplementation of vit D_3. These reports agrees with the work of Singh et al. (2003) and Mohammed *et al*. (1991), these authors reported that cholecalciferol supplementation of poultry diets may improve phytic acid-P utilization. Due to the reduced ileal P and increase in digestible and APD it can be concluded that phytic acid was effectively hydrolysed. Phytase supplementation has been reported to improve the amount of digestible P in plant feedstuffs and consequently reduce P loss from feed ingredient (Akinmusire and Adeola, 2009) as seen from the results of this study. There may also be an indirect effect of dietary Ca on P digestibility because vitamin D is activated at low Ca concentration, which may enhance both Ca and P absorption (Liem *et al.,* 2009 and Amerah *et al*., 2019), and therefore increase P digestibility. Also when the dietary concentration of Ca is low, with the addition of phyase, P digestibility increases (Poulsen *et al.,* 2010). The positive effect of phytase on P digestibility is in agreement with results from experiments in which pigs were fed diets based primarily on canola meal (Akinmusire and Adeola, 2009) and the increase in P digestibility is due to the release of P from phytate.

From the results, tibia ash which is a more sensitive indicator of mineral status and bone mineralization significantly increased when vit D_3 and phytase was added to the diets. This result agrees with the work of (Asadi *et al.,* 2023), the author used vitamin D_3 in the form of calcitrol and discovered a direct impact on the bone , improves mineralization of bones in young chicks, maintains balanced calcium and phosphorus levels in the organism, reduces an incidence of disorders of extremeties and tibial dyschondroplasia.

Conclusion and recommendation

Based on the results from this study, supplementation of cottonseed meal with Natuphos® phytase at 1000 units/kg of diet and vitamin D_3 at 10000 UI/kg fed with low calcium and phosphorus diet reduced ileal P and improved digested P and apparent P digestibility. Farmers, researchers and poultry farmers s should be encouraged to supplement poultry diets with phytase and vitamin D_3 as this will not only minimize eutrophication but decrease excessive dependence on inorganic phosphates in poultry.

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