



Effect of Phytase and Cholecalciferol Supplementation on the Digestibility of Phosphorus by Broiler Chickens Fed Cottonseed Meal and Rice Husk-Based Diets

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Abstract

This study was conducted to evaluate the effects of phytase and cholecalciferol supplementation in Cottonseed Meal (CM) and Rice Husk (RH)-based diets on the digestibility of phosphorus by broiler chickens (BC). In study 1 (n= 125) BC was divided into five treatments comprising a Positive Control (PC, D1) without CM and four CM-based diets: Negative Control (NC, D2), NC+vitaminD3 (D3), NC+phytase (D4) and NC+phytase+vitaminD3 (T5) replicated five times. In Study 2, (n=125) BC was also allotted to five treatments comprising a PC (D1) without RH and four RH-based diets: NC (D2), NC+vitaminD3 (D3), NC+phytase (D4) and NC+phytase+vitaminD3 (D5). In the CM-based diet, ileal phosphorus significantly (P<0.05) and reduced with a corresponding increase in the apparent phosphorus digestibility (APD) in D3. In the RH-based diets, APD was significantly (P<0.05) improved in D3. Phytase and vitamin D3 supplementation of CM and RH-based diets increased phosphorus digestibility in broiler chickens.

Keywords: Apparent Phosphorus Digestibility, Broiler Chicken, Cholecalciferol, Digestibility, Ileal Phosphorus Semi-Purified Diet.

Introduction

The bulk of the P in plant feedstuff is in the form of phytate (Ravindran *et al.*, 2008). Phytate is the main storage form of phosphorus in plants and it chelates nutrients that are required for phosphorus absorption (Humer *et al.*, 2015). Phytate is poorly utilized by poultry because they have restricted amounts of intestinal phytase. Hence, broiler feeds are augmented with inorganic P sources, and this results in huge quantities of P in the diet, which are afterward passed into the surroundings, producing eutrophication (Mutucumarana *et al.*, 2014). The actions of inherent phytase in diets for poultry and endogenous phytase in the digestive tract are not sufficient for the effective hydrolysis of phytate (Ilaboya and Iyayi, 2021). Dietary supplementation of phosphorus with exogenous phytase is effective in enhancing phosphorus digestion in poultry diets (Fan *et al.*, 2001). Usually, cholecalciferol has been expended in broilers' nutrition as a precursor for calcitriol. Kheiri and Landy (2019) compared the efficacy of 1- α (OH)D₃ alone or in a blend of microbial phytase on broiler quail's performance and excellence of tibia; the result specified positive interaction between 1- α (OH)D₃ and phytase on bone mineralization. This study aimed to examine the effects of phytase supplementation of broiler chickens fed diets comprising CM and RH to determine the effect of supplementing low P diets containing CM and RH with vitamin D₃ and phytase on P digestibility and retention in broiler chickens

Materials and Methods

The Ethics Committee on Research and Innovation of the University of Ibadan approved this research. This endorsement fit into the ethical standards placed down in the 1964 Declaration of Helsinki and its later amendment. Altogether birds were healthy during the experiment and no death was documented.

The test ingredients used in the project were CM and RH. Rice husk is a by-product of rice grains that are obtained by mechanical milling in dissimilarity to the solvent extraction method that is used for CM extraction.

Experimental Design

In experiment 1, at 20-day-old post-hatch, 125 broiler chickens were randomly allotted to five semi-purified diets comprising CM and RH as the test ingredient. For the CM diets, a 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) were formulated. In experiment 2 (RH diet n=125), a Positive Control (PC) diet with required NPP (4.84 g/kg diet) and a Negative Control (NC) diet in which the NPP is reduced by 45% (i.e. from 4.84 to 2.66 g/kg diet) was formulated. Three other diets each for CM and RH in which the NC diet is supplemented with either 1000 FTU phytase/kg diet, or 10,000 IU. vitamin D₃/kg diet or both was formulated, making a total of 10 semi-purified diets. On day 21, the chicks were independently weighed and allocated to the 10 diets in a randomized complete block design with 5 replicates of 5 birds each. Five cages were arbitrarily allotted to all of the 5 treatments (CM and RH respectively).

Table 1: Composition and nutrient content of cottonseed meal diets.

	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
Salt	2.50	2.50	2.50	2.50	2.50
Phytase (FTU/kg)	0.00	0.00	0.00	10.00	10.00
CC (g/kg)	0.00	0.00	0.25	0.00	0.25

TiO ₂	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
Non Phytate P (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

¹Composition of vitamin premix per kg; vit. A, 12500 I.U; vitamin E, 40mg; vitamin K₃, 2mg; vitamin B₁, 3mg; vitamin B₂, 5.5mg; niacin, 5.5mg; calcium pantothenate, 11.5mg; vitamin B₆, 5mg; vitamin B₁₂, 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, ²Phy=Phytase, ³ TiO₂=Titanium dioxide, CC=Cholecalciferol, PC=Positive Control diets, NC=Negative Control diets.

Table 2: Composition and nutrient content of rice husk diets.

	PC	NC	NC+VitD ₃	NC+ Phy	NC+Phy+Vit D ₃
Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Rice Husk` (RH)	0.00	300.00	300.00	300.00	300.00
Cassava Starch	608.00	378.00	377.75	368.00	367.75
Wheat gluten	250.00	200.00	200.00	200.00	200.00
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	18.00	18.00	18.00	18.00
Vitamin-Premix	2.50	2.50	2.50	2.50	2.50
Salt	2.50	2.50	2.50	2.50	2.50
Phytase (FTU/kg)	0.00	0.00	0.00	10.00	10.00
CC (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	3391.22	3390.35	3356.32	3355.44
CP (g/kg)	218.00	213.10	213.10	213.10	213.10
Ca (g/kg)	10.85	8.27	8.27	8.27	8.27
Total P (g/Kg)	4.84	6.50	6.50	6.50	6.50
NPP (g/kg)	4.84	2.66	2.66	2.66	2.66
Phytate P (g/kg)	0.00	3.84	3.84	3.84	3.84
Ca : NPP ratio	2.24	3.11	3.11	3.11	3.11
Ca : P ratio	2.24	1.27	1.27	1.27	1.27

¹Composition of vitamin premix per kg; vit. A, 12500 I.U; vitamin E, 40mg; vitamin K₃, 2mg; vitamin B₁, 3mg; vitamin B₂, 5.5mg; niacin, 5.5mg; calcium pantothenate, 11.5mg; vitamin B₆, 5mg; vitamin B₁₂, 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, ²Phy=Phytase, ³ TiO₂=Titanium dioxide, CC=Cholecalciferol, PC=Positive Control diets, NC=Negative Control diets

Table 3: Analyzed nutrient composition of cottonseed meal and rice husk

Nutrient	Cottonseed Meal	Rice Husk
Dry matter, g/kg	990	961
Crude protein, g/kg	334	227
Gross energy, kcal/kg	2200	2980
Calcium, g/kg	1.30	5.30
Total phosphorus, g/kg	7.75	4.90
Phytate phosphorus, g/kg	4.50	2.80
Non-phytate Phosphorus, g/kg	3.25	2.10

Table 4: Estimated nutrient densities in cottonseed meal-based diets (g/100g).

Nutrient	PC T1	NC T2	NC+Vit D ₃ T3	NC+Phy T4	NC+Phy+Vit D ₃ T5
Dry Matter	916	908.5	906.1	912.7	903.9
Crude Protein	205.5	192.5	218.0	203.5	201.0
Calcium	5.20	3.36	4.24	4.09	4.27
Total P	0.77	0.87	0.80	0.75	1.09
Gross Energy (Kcal/kg)	3256	3112	3122	3197	3322

CM: Cottonseed meal

Table 5: Estimated nutrient densities in rice husk-based diets (g/100g).

Components (g/kg)	PC D1	NC D2	NC+Vit D ₃ D3	NC+Phy D4	NC+Phy+Vit D ₃ D5
Dry Matter	905.4	910.9	906.9	908	912.6
Crude Protein	200.7	200.1	200.5	213.0	205.5
Calcium	8.19	6.05	8.19	5.49	6.20
Total P	1.83	1.76	1.79	1.90	1.77
Gross energy (Kcal/kg)	3456	3312	3321	3212	3235

RH: Rice husks

Feed intake was calculated as the difference between feed offered and refused on a cage basis throughout the eight-day feeding test. Birds were weighed at days 20 and 28 post-hatch on a cage basis to calculate body weight gain. On days 25 and 27 post-hatch, samples of fresh excreta were taken one time every day from trays placed underneath each cage. Day-to-day gatherings were combined on a replicate cage basis, bulked, and deposited in the freezer at -4°C. Samples were taken and air-dried in a force-draught oven at 55 °C for five days. On day 28, the birds were put to sleep by carbon (IV) oxide suffocation and divided to get digesta from the distal two-thirds of the ileum (Rodehutsord, 2012). Digesta samples were gotten by flushing the ileal content with deionized water. Samples from all replicate cages were pooled. The excreta and ileal samples were ground and deposited in air-tight sample bags at -4 °C.

Laboratory and Statistical Analysis

Dry matter of the excreta and ileal samples were dried at 105 °C for 24 hours in a pre-weighed dried crucible in an oven (method no 930.15) (AOAC, 2005). Samples were ashed and phosphorus concentration was obtained calorimetrically (UV) at 400 nm following digestion with nitric and perchloric acid. Titanium concentrations in the ashed samples of feed, excreta, and ilea were obtained by the colorimetric method succeeding digestion with concentrated tetraoxosulphate (VI) acid and absorbance read at 410 nm (Short, 1996).

Phosphorus intake was calculated as a concentration in grams of P intake per kilogram of DM. The quantity of digestible P was calculated from the intake of P and its digestibility, Apparent P retention (APR) was calculated as the amount of the P intake that was not rejected in the excreta.

Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, North Carolina, USA).

Results and Discussion

Ileal phosphorus significantly ($P<0.05$) reduced with an equivalent rise in the percentage apparent phosphorus digestibility in D3, while D4 and D5 did not differ from D1 in the two parameters (Table 6). There was a significant ($P<0.05$) reduction in excreta phosphorus and an equivalent rise in the APR in the PC diet and D5 when compared to the NC diet, D3 and D4.

Table 6: Phosphorus intake, phosphorus outputs, and calculated phosphorus response criteria of 28-day-old broilers¹ fed cottonseed meal-based diets

Measurements	PC D1	NC D2	NC+VitD ₃ D3	NC+Phy D4	NC+VitD ₃ +Phy D5	SEM
IP (g/kg DMI)	0.20 ^{ab}	0.26 ^a	0.05 ^c	0.08 ^{bc}	0.11 ^{bc}	0.25
APD (%)	76.61 ^b	75.81 ^b	94.61 ^a	90.64 ^{ab}	90.11 ^{ab}	2.53
ExP (g/kg,DMI)	0.20 ^c	0.78 ^a	0.55 ^b	0.56 ^b	0.51 ^b	0.04
APR (%)	76.99 ^a	26.18 ^c	37.67 ^c	35.63 ^c	54.38 ^b	4.00

^{a b c} Means in a row with different superscripts are meaningfully dissimilar from each other ($P<0.05$), ¹ IP: Ileal phosphorus, APR: Apparent Phosphorus Retention, ExP: Excreta P, PC= Positive control, NC: Negative control, ²Standard error of mean, DMI: dry matter Intake

Table 7 shows that there was no observable ($P>0.05$) difference in ileal P and APR across all the treatments. Apparent digestible phosphorus in D3 compared favorably with the PC diet. Excreta phosphorus was significantly ($P<0.05$) reduced in the supplemented diets (D3, D4, and D5) and the PC diet when compared with the NC diets.

Table 7: Phosphorus intake, phosphorus outputs, and calculated phosphorus response criteria of 28-day-old broilers fed rice husk-based diets

Measurements	PC D1	NC D2	NC+VitD ₃ D3	NC+Phy D4	NC+VitD ₃ +Phy D5	SEM
IP (g/kg DMI)	0.11	0.13	0.14	0.14	0.21	0.02
APD (%)	80.78 ^{ab}	79.53 ^{ab}	90.88 ^a	75.97 ^{ab}	67.45 ^b	2.82
ExP(g/kg DMI)	0.39 ^b	0.94 ^a	0.55 ^b	0.48 ^b	0.42 ^b	0.05
APR (%)	31.08	21.24	28.12	19.83	36.64	3.51

^{abc} Means in a row with different superscripts are meaningfully dissimilar from each other ($P<0.05$), PC: Positive control, NC: Negative control. ²Standard error of mean, DMI: Dry Matter Intake

It has been reported that phytase supplementation enhances Ca and P retention in broiler chickens (Rodehutsord *et al.*, 2012) and digestible P in pigs, with a decrease in excreted P by 21.5% (Dilger and Adeola, 2006). The results obtained in this research enhanced ileal digestible P and an equivalent P retention in both experiments agreed with these previous findings.

Increases of APD were highest in the CM diet compared with values gotten for RH. It can also be accredited to the presence of suitable substrate for microbial phytase action (Dersjant-Li, *et al.*, 2014). The modification in processing methods used to produce CM and RH possibly brought about fluctuating amounts of phytate in their by-products. Hence better substrate-to-enzyme concentration might have elicited the perceived rise in APD, a reduction in ilea P and excreta P.

The results from the two experiments show that irrespective of the assayed feedstuff, there was a difference in the effect of phytase and vit D₃ on P digestibility. Comparable effects on ileal P digestibility and retention were documented by (Leytem, 2008), who documented a rise in the hydrolysis of phytate P in a study with corn, wheat, barley, and oats in broiler chickens as a consequence of the totaling of 1000 phytase units to the diets. Phytase supplementation has also been stated to improve digestible P in pigs with a decrease in excreta P by 21.5% (Harper,

1997). These findings support the outcome of this study of an enhanced ileal digestible P and an equivalent P retention.

Ileal phosphorus significantly ($P<0.05$) reduced with an equivalent rise in APD, and APR and a significant ($P<0.05$) decrease in excreta P with the supplementation of the combination of vit D₃ and phytase in the CM diets. In the RH diets, ileal P was reduced with an equivalent rise in APD in D3. Supplementation of vit D₃ and phytase in combination did not have any effect on APR and excreta output. Phytase and vit D₃ supplementation has been documented to increase the quantity of digestible P in plant feedstuffs and consequently reduce P loss from feed ingredients (Akinmusire and Adeola, 2009; Iyayi *et al.*, 2013) as seen from the results of these studies.

Conclusion and Recommendation

Supplemental phytase and vitamin D₃ improved Apparent Phosphorus Digestibility and reduced ileal P in cottonseed meal-based diets. However, from the results, there was a high percentage increase in APD and a reduction in ileal phosphorus which was observed in phytase and vit D₃-supplemented cottonseed meal diets than in rice husk diets. Cognizance of supplementing poultry diets with phytase and vit D₃ has to be intensified because the use of exogenous phytase and vit D₃ would not only reduce eutrophication but also reduce reliance on inorganic phosphates in poultry.

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Contribution by Authors

Equal contribution. All authors declared that ‘written informed’ consent was obtained from the approved parties for the publication of this article and accompanying images.

Conflict of Interests

There is no conflict of interest.

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