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Research Article

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-day-old Arbor Acre 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. Between days 25 and 27 post-hatch, samples of fresh excreta were collected once daily from each cage. On day 28, the birds were euthanized by carbon (IV) oxide asphyxiation and dissected to obtain and digesta from the 2/3rd of the ileum. All data were analysed using the GLM procedure in SAS. 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 (5 g/Kg) was included as marker. 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 low calcium and phosphorus diets supplemented with phytase and vitamin D3 on cottonseed meal improved digestible P in broiler chickens.

Keywords: Cottonseed Meal; Phytase; Vitamin D3; Ileal Phosphorus; Broilers

Abbreviations

CC: Cholecalciferol; Ca: Calcium; SAS: Statistical Analysis Software; PC: Positive Control; NC: Negative Control; CSM: Cottonseed Meal; TiO_2: Titanium Dioxide; DP: Digestible Phosphorus; IDP: Ileal Digestible Phosphorus; APD: Apparent Phosphorus Digestibility

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 [1]. The level of Ca in the diet may also affect the utilization of phytic acid-P through the formation of insoluble Ca-phytate and/or reduction of phytase activity [2]. Cholecalciferol (vitamin D_3) plays a role in Ca and P absorption, and therefore influences their utilization [3]. 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 [4,5]. Vitamin D_3 is best known for its role in Ca and P metabolism in skeletal development and maintenance, as well as egg shell strength. Calcium to phosphorus ratio and phosphorus content of diets also influence the inorganic P release from phytate by phytase [6]. Animal feed contains high amount of calcium, calcium content in diet can have a large impact on phytate P utilization and phytase efficiency [5]. Increasing the Ca to total P ratio may have negative impact on phytase activity [7]. The authors [7] 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 or poultry. Similarly [8], 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% [9], 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

This study was carried out at the poultry unit of the Faculty of Agriculture and Agricultural Technology Research Farms, Benson Idahosa University, Benin City, Edo State.

Experimental animals and experimental diets

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 20days and later replicated in cages. Pans were placed beneath each cage for fresh excreta collection daily. Positive control (PC) diet with required Non Phytate Phosporus (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 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 [10] after asphyxiation with CO_2. Natuphos® phytase (3-phytase derived from *Aspergillus niger*) was used as the exogenous phytase (5g/kg). The trial lasted 8days.

Management

Caged birds were housed in an environmentally controlled Room. Fresh water and feed were available *ad libitum* throughout the experiment. Feed intake was calculated as the difference in amounts offered and refused on cage basis during the 8-day feeding trial.

Feed intake = feed offered – refusals

Birds were weighed at days 20 and 28 post hatch to calculate body weight gain. The initial live weights of the chicks were measured and recorded at the beginning of the experiment and the final weight at the end of the experiment using a digital weighing scale. Weight gain of the bird was gotten by subtracting the initial live weight from the final live weight of the bird.

Weight gain = final weight – initial weight

Chemical analysis

Dry matter (DM) was determined by drying excreta and ileal samples at 105ºC for 24h in a pre-weighed dried crucible in a convention oven [11]. Samples were ashed and phosphorus concentration was determined colorimetrically at 400nm following digestion with nitric and perchloric acid. Calcium was determined by colorimetric assay following digestion of the ashed samples with 6M HCl to release Ca [11] (AOAC International, 2005). Titanium concentrations in the ashed samples of feed, excreta and ileal were determined by colorimetric method following digestion with concentrated sulphuric acid and absorbance read at 410nm as described by [12] Short., *et al*. (1996). Crude protein of the test ingredients, experimental diets, ileal and excreta samples was determined by total combustion method [11] (AOAC International, 2005). Gross energy of the test ingredients and experimental diets was determined by adiabatic bomb calorimetry, standardized with benzoic acid. Crude fat content of test ingredients was determined by Soxhlet extraction [11] (AOAC International, 2005)

Statistical analysis

Data were analysed using the GLM procedure of SAS (13) (SAS Institute, 2012). Duncan Multiple Range Test method was used to identify statistically different means ($P < 0.05$). The following parameter was calculated for using the following formular

AID = 100 – (ileal nutrient × marker in diet/nutrient in diet × ileal marker)

ATTR = 100 – (marker in feed × nutrient in feces /marker in feces × nutrient in feed)

Results and Discussion

Table 1 shows the gross composition of experimental diets fed to the broiler chickens, expressed in g/kg. The proximate analysis and DM (916, 908.5, 906.1, 912.7 and 903.9) content of the experimental diets are shown in Table 2. The analysed concentrations of total P were; 0.77, 0.87, 0.80, 0.75, 1.09% and Ca; 5.20, 3.36, 4.24, 4.09, 4.27% in CSM diets. Analysed values of CSM for crude protein, were; 20.55, 19.25, 21.80, 20.35 and 20.10 %. Analysed energy (Kcal/kg) values was highest in the NC diets supplemented with a combination of phytase and vitamin D_3 (3322Kcal/kg) and lowest in the negative control diets (3122 3322Kcal/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
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
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

Table 1: Gross composition of experimental diets.

[1]Composition of vitamin premix per kg of diet: vitamin 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, [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

Parameter	PC T1	NC T2	NC + Vit D3 T3	NC + Phy T4	NC + Phy + Vit D_3 T5	[2]SEM
Feed intake (g/bird)	225.80	243.16	218.44	234.28	203.00	6.41
DMI (g/bird)	213.70^{ab}	224.48^{a}	204.23^{ab}	220.82^{ab}	176.96^{b}	6.16
BWG (g/bird)	57.16^{b}	54.72^{b}	72.12^{a}	77.34^{a}	67.20^{ab}	2.55
Feed conversion ratio	3.99^{a}	4.58^{a}	3.05^{b}	3.10^{b}	3.00^{b}	0.18
Tibiae bone ash (%)	20.88^{b}	25.83^{a}	24.67^{ab}	27.31^{a}	21.15^{b}	0.73

Table 4: Selected growth performance indices and percentage tibiae bone ash of 28-day-old broilers1 fed cottonseed meal-based diets.

[a b c] Means in a row with different superscripts are significantly different from each other (P < 0.05).

[1]Each value represents mean of 5 replicates (5 birds/replicate), PC: Positive control, NC: Negative control

[2]Pooled standard error of mean, DM: Dietary dry matter content, DMI: dry matter Intake, BWG: Body weight gain

Table 3 shows the results for Ileal, digested and apparent phosphorus digestibility of broilers fed cottonseed meal-based diets. Ileal phosphorus in the diet supplemented with vitamin D3 (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 D3 (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.

Parameters	PC T1	NC T2	NC + VitD_3T3	NC + Phy T4	NC + Phy + VitD_3 T5	SEM
P (g/kg of diet)	0.87^{e}	1.06^{b}	0.88^{c}	0.87^{d}	1.11^{a}	0.21
IP (g/kg DMI)	0.20^{ab}	0.26^{a}	0.05^{c}	0.08^{bc}	0.11^{bc}	0.25
DP (g/kg DMI)	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

Table 3: Ileal, digested and apparent phosphorus digestibility of 28-day-old broilers fed cottonseed meal-based diets.

[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, IP- Ileal phosphorus, DP- Digested phosphorus, APD- Apparent phosphorus Digestibility

Results of selected growth performance indices investigated and percentage tibiae bone ash of the experimental birds fed CSM-based diets are presented in Table 4. Feed intake and dry matter intake of the birds had no significant (P > 0.05) response to feeding the treatment diets. Body weight gain improved significantly (P < 0.05) as the NC diet was supplemented with vitamin D3 (T3) and phytase (T4) when compared to the PC (T1) treatment. Feed conversion ratio was also significantly (P < 0.05) improved in the supplemental diets in comparison with the PC treatment. 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.

Results for calcium intake and digestibility of birds fed cottonseed meal-based diets are presented in table 6. The analysed dietary calcium concentrations in all diets differ significantly (P < 0.05). Due to the relationship between calcium and phosphorus, calcium: total phosphorus ratio was also calculated across diets using the analysed values. Ileal calcium in T3 was not significantly (P < 0.05) different from the PC (T1) but T4 and T5 differ (P < 0.05) from the PC diet. Digested calcium and precaecal calcium in the supplemented diets differ significantly (P < 0.05) from the PC diet. Percentage apparent calcium digestibility in the PC (T1) diet and T3 significantly (P < 0.05) increased when compared to the diet supplemented with phytase (T4).

Components	PC T1	NC T2	NC+Vit D_3T3	NC+Phy T4	NC+Phy+Vit D_3T5
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

Table 4: Analysed nutrient composition of cottonseed meal-based diets (g/100g).

[1]L: Linear Effect,[2]Q: Quadratic Effect, BWG: Body Weight Gain, FI: Feed Intake, FCR: Feed Conversion Ratio

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 [4] Liem., *et al.,* 2009 and [5] Sylwester., *et al.,* 2016. 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 [14], 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 [5], and therefore increase P digestibility. Also when the dietary concentration of Ca is low, with the addition of phyase, P digestibility increases [3,15]. The positive effect of phytase on P digestibility is in agreement with results from experiments in which broiler chickens were fed diets based primarily on cottonseed meal and rice husk respectively [16] 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 [17], 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

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 Agricultural firms 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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Conflict of Interest

There was no conflict of interest.

Bibliography

1. Asadi ZK., *et al*. "Interactive effects of calcium, vitamin D_3 and exogenous phytase on phosphorus utilization in male broiler chickens from 1 to 2 days post-hatch: A meta-analysis approach". *Animal Feed Science and Technology* 295 (2023): 15546-43656.

2. Shi CX., *et al*. "High doses of phytase alleviate the negative effects of calcium and phosphorus imbalance on growth performance and bone mineralization in broiler chickens". *Brazilian Journal of Poultry Science* 24 4 (2022): 1-9.

3. Khan SH., *et al*. "Effect of level of cholecalciferol supplementation of broiler diets on the performance and tibial dyschondroplasia". *Journal of Animal Physiology animal Nutrition (Berl)* 94.5 (2010): 584-593.

4. Liem A., *et al*. "Experimental approach to optimize phytate phosphorus utilization by broiler chickens by addition of supplements". *Poultry Science* 88 (2009): 1655-1665.

5. Slywester S., *et al*. "Effect of dietary vitamin Dand its metabolites in poultry- review and implications of the recent studies". *Worlds Poultry Science Journal* 73 1 (2002): 57-68.

6. Sommerfeld V., *et al*. "Interactive effects of phosphorus, calcium, and phytase supplements on products of phytase degradation in the digestive tract of broiler chickens". *Poultry Science* 97.4 (2018): 1177-1188.

7. Caroline B., *et al*. "Cacium:phosphorus ratios and supplemental phytases on broiler performance and bone quality". *Journal of Applied Poultry Research* 29.3 (2020): 584-599.

8. Liu JB., *et al*. "Phosphorus digestibility response of broiler chickens to dietary calcium to phosphorus ratios". *Poultry Science* 92 (2013): 1572-1578.

9. Ravindran V., *et al*. "Total and phytate phosphorus contents of various foods and feedstuffs of plant origin". *Food Chemistry* 5.2 (1996): 133-136.

10. Rodehutscord M., *et al*. "A note on sampling digesta from the ileum of broilers in phosphorus digestibility studies". *Poultry Science* 91 (2012): 4.

11. AOAC International. "Official Methods of Analysis 18th edition". AOAC International, Washington, DC (2005).

12. Short FJ., *et al*. "Determination of titanium dioxide added as an inert marker in chicken digestibility studies". *Animal Feed Science and Technology* 59: (1996) 215-221.

13. SAS (Staitistical Analysis System) Users Guide: Statistics. SAS Institute Inc. Cary, NC.

14. Akinmusire AS and Adeola O. "True digestibility of Phosphorus in Canola and Soybean meal for growing pigs: Influence of microbial phytase". *Journal of Animal Science* 87: (2009) 977-983.

15. Poulsen HD., *et al*. "Phosphorus digestibility is highly influenced by phytase but slightly by calcium in growing pigs". *Livestock Science* 134: (2010) 100-102.

16. Ilaboya Ibinabo I and Iyayi Eustace A. "True phosphorus digestibility of cottonseed meal and rice husk supplemented with microbial phytase in broiler chickens". *South African Journal of Animal Science* 51 (2021): 104-112.

17. Seed A., *et al*. "Cumulative effect of phytase and vitamin D supplementation on performance and bone mineralization in broiler". *Eurasian Journal of Veterinary Science* 31.2 (2015): 102-108.