TRUE DIGESTIBILITY OF PHOSPHORUS BY ROSS 308 BROILER CHICKENS FED DRIED CABBAGE MEAL DIETS SUPPLEMENTED WITH EXOGENOUS PHYTASE

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Abstract
One hundred and forty-four 21- day- old broiler chicks (BCs) were assigned to the six experimental diets in a 2 x 3 factorial arrangement with six replicates of eight birds each. Six dried cabbage meal (DCM)-based semi-purified diets (100, 200, and 300g/kg) containing three levels of Total Phosphorus (TP, g/Kg): 2.85, 3.50, and 4.15 with or without phytase (1000 units/Kg) were formulated. Titanium dioxide (5 g/Kg) was included as a marker in the experiments. The BCs were fed for eight days, and fresh excreta were collected from day's five to seven. On day eight, the ileal digesta was harvested after slaughtering. Ileal EPL (Endogenous Phosphorus Loss), total tract EPL, TIPD (True Ileal Phosphorus Digestibility), and True Phosphorus Retention (TPR) were estimated. Data were analyzed using descriptive statistics, linear regression. Total Tract Phosphorus retention of phytase-supplemented diets was 1.28, 1.51, and 1.88, which were higher ($P<0.05$) than those without phytase (1.26, 1.53, and 1.68), except for diets with 200g/kg inclusion of DCM. Estimated ileal EPL for birds fed phytase-supplemented and un-supplemented diets (g/kg DMI) were 0.025 and 0.8807, respectively. Phytase reduced ($P<0.05$) ileal EPL and total tract EPL by 85.57% and 13.09%, respectively. Supplementation with phytase significantly ($P<0.05$) increased TIPD and TPR by 42.37% and 20.57 respectively. Phytase supplementation of DCM-based diets improved ($P<0.05$) total tract phosphorus retention, true ileal phosphorus digestibility, and reduced ($P<0.05$) endogenous phosphorus losses in broiler chickens.

Keywords: Dried cabbage meal, titanium dioxide, ileal digesta, endogenous phosphorus loss, true phosphorus digestibility

Description of Problem

Phosphorus (P) utilization by poultry depends on the form of dietary phosphorus, as phosphorus in phytate form found in plant feedstuffs is poorly available (1). When phosphorus is provided in the form of phytate, dietary allowances are typically in excess to ensure that the animals' requirements are met (2). In these circumstances, large amounts of phytate P are excreted and can pose an environmental risk to soil and aquatic systems when manure is applied to land (3). The strong chelating potential of phytate often compromises the absorption of other minerals, such as calcium, zinc, magnesium, and copper including protein and energy utilization (4, 5), thereby affecting the role of phosphorus in most metabolic processes and bone development. These minerals are also essential in animal nutrition due to their involvement in hormonal and enzymatic systems, as well as bone development. If their supply falls below the required levels, negative effects on bone development and growth can be observed because plant-based feedstuffs commonly used in broiler diets do not provide adequate concentrations of available phosphorus, zinc, and copper for the birds. Hence, their nutrients are usually supplemented with external sources of these minerals. The concentration of these minerals used by the industry in broiler diets is often higher because they enhance growth performance and are supplemented with large safety margins to account for dietary antagonists such as phytate. Available phosphorus is limited in Plant-Derived Feed Ingredients (PDFIs) and are characterized by low True Ileal Phosphorus Digestibility (TIPD) and high Endogenous Phosphorus Loss (EPL) with negative environmental consequences. The potential of exogenous phytase to break down Phytate-Phosphorus Complex (PPC) has been documented. However, information on TIPD and EPL in BCs fed diets supplemented with phytase is scarce. Therefore, the effects of phytase supplementation in dried cabbage meal (DCM)-based diets on TIPD and EPL in BC were investigated.

Materials and Methods

The study was conducted at the Faculty of Agriculture and Agricultural Technology Research Farms, Benson Idahosa University, Benin City, Edo State. The test ingredient used in this study is dried cabbage meal (DCM). Fresh cabbage leaf residues were collected, air-dried, and crushed using a hammer mill.

Table 1. Gross composition of experimental diet

	0 FTU/Kg (Phytase)			1000FTU/Kg(Phytase)		
Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Dried Cabbage Meal (DCM)	100.00	200.00	300.00	100.00	200.00	300.00
Cassava Starch	522.50	420.75	319.00	512.50	410.75	309.00
Casein	220.00	220.00	220.00	220.00	220.00	220.00
Soya oil	10.00	10.00	10.00	10.00	10.00	10.00
Dextrose	102.25	102.25	102.25	102.25	102.25	102.25
Methionine	3.00	3.00	3.00	3.00	3.00	3.00
Lysine	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	10.25	12.00	13.75	10.25	12.00	13.75
Vitamin-Premix	2.50	2.50	2.50	2.50	2.50	2.50
Salt	2.50	2.50	2.50	2.50	2.50	2.50
Phytase Enzyme	0.00	0.00	0.00	10.00	10.00	10.00
Titanium Dioxide	25.00	25.00	25.00	25.00	25.00	25.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated Nutrients						
ME Kcal/Kg	3958.51	3909.13	3859.74	3923.61	3874.23	3824.84
CP (g/kg)	205.64	219.44	233.24	205.64	219.44	233.24
Ca (g/kg)	5.37	6.16	6.96	5.37	6.16	6.96
Total P (g/Kg)	2.85	3.50	4.15	2.85	3.50	4.15
Phytate P (g/Kg)	0.43	0.86	1.29	0.43	0.86	1.29
Non Phytate P (g/kg)	2.42	2.64	2.86	2.42	2.64	2.86
Ca : NPP ratio	2.22	2.33	2.43	2.22	2.33	2.43
Ca : P ratio	1.88	1.76	1.68	1.88	1.76	1.68

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; Phytase premix prepared by mixing phytase with maize. 3Titanium dioxide premix prepared by mixing 1g of titanium oxide with 4g of maize

Experimental animals, feeding and management

One hundred and forty four-day-old Ross 308 broiler chicks were fed commercial broiler starter diet that met (6) nutrient requirements for broiler chicks for 20 days. At day 20 post-hatch, 144 birds were transferred to metabolic cages, tagged, individually weighed and randomly allotted to experimental diets, with six replicates in a randomized complete block design. Birds were provided with free access to water and experimental diets for eight days.

Dietary Treatments

Six semi-purified diets containing 100, 200 and 300g of DCM/kg (table 1) with or without 1000 units of phytase/kg diet were formulated. The dietary inclusion levels of the test ingredients were obtained by the gradual replacement of cassava starch with the CM. Soya oil were added to the diets to obtain similar gross energy across the diets. Across all studies, varied dietary levels of limestone were added to the experimental diets to ensure that similar calcium: phosphorus ratios were maintained. Titanium dioxide was included at the rate of 5g/kg of diet as an indigestible marker.

Collection of Digesta and Excreta Samples
Between days 25 and 27 post-hatch, samples of fresh excreta were collected daily from pans placed beneath the cages. Daily collections were pooled on a replicate cage basis, bulked, and stored. On day 28, the birds were slaughtered and dissected to obtain digesta from the distal two-third of the ileum using the procedure of (7). Digesta samples were obtained by flushing the ileal content with deionised water. Excreta and ileal samples from each replicate cage were pooled

Statistical Analysis
Data was analyzed using the GLM procedure of Statistical Analysis Software (8). Orthogonal polynomial contrasts were employed to ascertain the effects of graded levels of P on all response criteria, and a level of 0.05 was considered significant. The experimental design followed a randomized complete block design with 2x3 factorial arrangements. The statistical linear regression model used is PD = (TPD×PI) ± EPL, Where PD represents ileal digested P or retained P, which is the difference between dietary P intake and ileal or excreta P output; EPL represents the intercept of the regression curve, PI represents P intake; True phosphorus digestibility (TPD) is the slope of the regression curve which represents an estimate of true ileal digestibility or true P retention. Slopes of the regression was used to compared within sampling sites (ileal and excreta) with or without phytase using student's t-test.

Results and Discussion
Phytase supplementation reduced ileal P output significantly ($P<0.05$) at the inclusion of 200g/kg DCM in the diet. The values of ileal P outputs for birds fed DCM without supplemental phytase were 0.32, 0.76, and 0.34 g/kg DM intake of diet (DMI), while birds fed diets supplemented with phytase , were 0.24, 0.16, and 0.53 g. Phytase and its interaction with P (phosphorus × phytase) had a significant effect ($P<0.05$) on ileal P output. The excreta P output of the birds was not affected ($P>0.05$) by the increasing intake of P from DCM, the addition of phytase, or the interaction phosphorus × phytase. The excreta P output values for birds fed DCM without phytase were 0.42, 0.28, and 0.43 g/kg DMI, while birds on phytase-supplemented DCM diets, were 0.47, 0.33, and 0.22 g per kg DMI. The value was lower at 300 g/kg inclusion of DCM with phytase supplementation. Ileal digested P reduced significantly ($P<0.05$) on all response criteria. It was also observed that a sequential increase in DCM in the diet resulted in a significant ($P<0.05$) increase in phosphorus retained by the birds.

Table 2: Phosphorus intake, phosphorus outputs and calculated phosphorus response criteria of 28-day-old broilers fed cabbage meal-based diets

Parameter	Phytase (0 FTU/kg)			Phytase (1000 FTU/kg)				P-value		
	DCM 100g/kg	DCM 200g/kg	DCM 300g/kg	DCM 100g/kg	DCM 200g/kg	DCM 300g/kg	Pooled SEM	Phy	DCM	Phy*DCM
Dietary P intake g/kg of diet	1.68	1.81	2.11	1.75	1.84	2.10	_	_	_	_
Ileal Poutput (g/kg DMI)	0.32	0.76	0.34	0.24	0.16	0.53	0.10	0.05	0.16	0.004
Digested P (g/kg DMI)	1.36^{a}	1.04^{ab}	1.77^{ac}	1.51^{a}	1.68^{ab}	1.57^{ac}	0.10	0.03	0.02	0.003
Apparent P Digestibility (%)	81.10	57.70	84.00	86.22	91.22	74.81	4.73	0.03	0.19	0.002
Excreta P	0.42	0.28	0.43	0.47	0.33	0.22	0.08	0.58	0.21	0.20

(g/kg DMI)										
Retained P	1.26^{a}	1.53^{b}	1.68^{c}	1.28^{a}	1.51^{b}	1.88^{c}	0.08	0.31	<0.01	0.37
Apparent P Retention (%)	74.81	84.62	79.47	73.16	81.91	89.58	4.30	0.60	0.05	0.29

*abc Means in a row with different superscripts are significantly different from each other (P<0.05) 1Each value represents the mean of 3 replicates (6 birds/replicate), Pooled standard error of mean, DM= dietary dry matter content, DMI = dry matter intake,*P-Phosphorus*

Retained P was higher for birds with the inclusion of 300 g DCM/kg, with the presence of phytase in the diet. The percentage apparent P digestibility (APD), For birds fed DCM without and with phytase was 81.10, 57.70, and 84.0% compared to 86.22, 91.22, and 74.81% at 100, 200, and 300 g DCM/kg diet, respectively. Phytase and the phosphorus × phytase interaction had a significant influence (P<0.05) on the APD of the birds. However, only P from DCM elicited a significant (P<0.05) response for the apparent P retention (APR) of the birds. On an average basis, birds on phytase-supplemented 300 g/kg DCM diets had higher APR than those on phytase-unsupplemented DCM diets at the same level of inclusion. The results of the studies indicate that phytase supplementation of the respective test ingredients led to either a numerical or statistical increase in ileal digested P and retained P. The observed increase in ileal digested and retained P demonstrates the phytase effect in breaking down phytate-phosphorus complexes, leading to a consequent rise in ileal phytate-P disappearance (9). Phytase supplementation has been reported to enhance the amount of digestible P in plant feedstuffs, subsequently reducing P loss from feed ingredients (10), as indicated by the results of this study. The true total tract P retention (TTTPR) coefficients were 0.887 for DCM without phytase and 0.967 with phytase. Endogenous P flow in excreta were 0.167 g/kg for diets without phytase supplementation and 0.036 g/kg for those with supplementation (Table 3).

Table 3. Linear relationship of P intake, digestion, and retention in ROSS 308 broilers.

Item	Regression Equation	R^2	Estimated EPL (g/kgDM)	P-value	
Ileal section					**Estimated TIPD**
DCM	y = 0.218x - 0.8807	0.4451	0.8807	0.001	21.8^{a}
DCM+Phytase	y =0.4237x + 0.025	0.79	0.025	0.001	42.37^{b}
Total Tract Section					**Estimated TPR**
DCM	y=0.8865x - 0.1666	0.5683	0.1666	0.001	88.65^{a}
DCM+Phytase	y=0.9689x - 0.0357	0.6803	0.0357	0.001	96.81^{b}

Regression of digested or retained P against dietary P intake, TIPD:True Ileal Phosphorus Digestibility, TPR:True P Retention

Phytase supplementation increased true ileal P digestibility (TIPD) of DCM, with estimated TIPD values of 42.37%. Phytase supplementation resulted in a 20.57% increase in TIPD for DCM. Simultaneously, the supplementation of DCM with phytase increased the estimated TPR values to 96.81%. This increase translated to 8.16% percentage increase. Akinmusire and Adeola (10) reported an improvement in TPD of canola and soybean meal with the addition of phytase to diets for growing pigs. Strong linear relationships was observed in this study between ileal digested P, retained P, and dietary P intake for the test ingredient. Other authors have reported a strong linear relationship between digesta P outputs and dietary P intake (11). Phytase supplementation has also been associated with improved Ca and P retention in broiler chickens (12) and increased digestible P in broilers, resulting in a 3.5% to 4.2% reduction in ileal P by the broiler chickens (12). These reports support the results in this study of improved ileal digestible and retained P.

Conclusion and Application

Phytase supplementation of DCM-based diets improved total tract P retention, true ileal P digestibility, and reduced endogenous phosphorus losses in ROSS 308 broiler chickens. Phytase supplementation in DCM-based diets enhances phosphorus utilization and growth efficiency in ROSS 308 broilers while reducing feed costs and environmental phosphorus output, making it a practical strategy for both sustainable research innovation and commercial poultry industry adoption.

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