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Estimation of true preceacal phosphorus digestibility of phytase supplemented groundnut cake in broiler chicken

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Abstract— *An experiment was conducted to investigate effect of phytase supplemented groundnut cake (GNC) on endogenous preceacal phosphorus loss (EPPL) and true preceacal phosphorus digestibility (TPPD) in broiler chickens using regression technique. A total of 300, one-day-old, unsexed broiler chickens were raised on standard commercial starter diet. At day 20, selection for 216 male broilers chicks was made and chicks were completely randomized and allotted to 6 dietary treatments in 3×2 factorial arrangement with 3 levels of P (1.40, 2.66 or 3.96 g/kg diet) obtained from varying proportions of GNC and 2 levels of microbial phytase (0 FTU/kg or 1000 FTU/kg), feeding trial lasted for 7 days. With inclusion of chromic oxide in experimental diets, the index method (Adeola, 2001) was used to calculate apparent preceacal phosphorus digestibility (APPD). Generated regression curve obtained from the relationship between preceacal digested P and dietary P intake was used to calculate EPPL and TPPD. Results showed that increasing P intake had linear effect ($p<0.05$) on body weight gain and feed to gain ratio. Neither P, phytase nor their interaction affected ($p>0.05$) feed intake, dry matter intake and tibia ash of the birds. Addition of phytase resulted in 44.1% reduction in preceacal P output, while levels of P, phytase and interaction linearly increased ($p<0.05$) digested P and retained P from GNC. From the regression curve, phytase improved TPPD of groundnut cake in birds by 34.48% with a 52.27% reduction in EPPL. True preceacal P digestibility of GNC in 28 days-old, broiler chickens improved with 1000units phytase supplementation of GNC*

Keywords— ***preceacal, digestibility, endogenous, phosphorus, groundnut cake.***

I. INTRODUCTION

Oil seed meals (OSM), which make up 25 to 35% of formulated diets for poultry feed usually, have high protein and P contents. Apart from soyabean meal, a closely ranked OSM commonly used in poultry feed is groundnut cake (GNC), a by-product obtained after oil extraction from groundnut seeds. According to Raboy (1997), about 65-80% of P in vegetative feed ingredients is bound as phytate-P complex. The poor utilisation of complexed P tends to limit amount of P from OSM available to poultry birds. Sequel to this, strategies have been documented to further improve available P from OSM for use by birds. One of such strategies have led to the need for continuous measure of available P in poultry feed ingredients (Rodehutscord,2009; Mutucumarana, 2014) and results expressed on "apparent digestibility" basis, without accounting for endogenous P loss. Although, endogenous loss of nutrients is well documented as an inevitable loss from the animal but contribution to such loss from dietary origin cannot be ignored and needs to be accounted for. According to Butt *et al,* (1993), ingredient specific factors constitute a portion of endogenous loss of nutrient. To circumvent this limitation, Currently, expressions for P is being canvassed on "true digestibility" basis. To this end, techniques that simultaneously estimate TPPD, true total tract retention and EPPL for P have been reported; radioactive labelled isotope technique (Al-Masri, 1995),

regression approach (Fan *et al*., 2001, Adeola and sands, 2006) and P-free diet method (Petersen and Stein, 2006).

The use of exogenous phytase to make more P available from phytate-P complex is another known strategy. To a large extent findings from research conducted on poultry and pigs have demonstrated benefits of microbial phytase to include improved digestibility of P, calcium and Zinc (Paiva *et al*., 2014). Studies of (Fan *et al*., 2001; Ajakaiye *et al*., 2003; Dilgner and Adeola, 2009; Iyayi *et al*., 2013; Mutucumarana, 2014). estimated TPPD, true total tract P retention and EPPL of feed ingredients in poultry and pigs. However, investigation on TPPD from phytase supplemented-GNC in broiler chicks is limited. Therefore, this study aims to estimate EPPL and TPPD of 28-day-old broiler chickens fed phytase supplemented GNC.

II. METHOD

2.1 Experimental diets

A total of six semi-purified diets containing; 210, 420 or 630g of GNC, with or without phytase at dose of 1000 *FTU*/kg were formulated (table 1). Adjustment for calcium: P ratio and energy were made using limestone and soy oil, respectively. Chromic oxide (Cr_2O_3) at a rate of 5g/kg of diet served as indigestible marker. Exogenous phytase was BASF Natuphos® phytase (3-phytase derived from *Aspergillus niger*) and inclusion was based on dosage recommendation to obtain dose rate of 1000 FTU/kg.

Table1. Gross composition of experimental diets (g/kg) (as-fed basis)

	PHYTASE (0 FTU/ Kg diet)			PHYTASE (1000 FTU/ Kg diet)		
Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
GNC	**210.00**	**420.00**	**630.00**	**210.00**	**420.00**	**630.00**
Cassava Starch	**588.50**	**334.20**	**105.90**	**578.50**	**324.20**	**95.90**
wheat gluten	130.00	130.00	130.00	130.00	130.00	130.00
Soy oil	2.00	44.00	60.00	2.00	44.00	60.00
Dextrose	35.00	35.00	35.00	35.00	35.00	35.00
Methionine	1.00	1.00	1.00	1.00	1.00	1.00
Lysine	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	**2.50**	**4.80**	**7.10**	**2.50**	**4.80**	**7.10**
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
[1]Phytase premix	0.00	0.00	0.00	10.00	10.00	10.00
[2]Chromium oxide Premix	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 (g/kg)						
ME (Kcal/Kg)	3173.21	3126.75	2964.13	3138.31	3091.85	2911.23
Crude protein	200.13	294.63	389.13	200.13	294.63	389.13
Calcium (Ca)	1.44	2.76	4.07	1.44	2.76	4.07
Phosphorus (P)	1.40	2.66	3.96	1.40	2.66	3.92
Ca : P (ratio)	1.03	1.04	1.04	1.03	1.04	1.04

[1]Phytase premix prepared by mixing phytase with maize. [2]Chromic oxide premix prepared by mixing 1g of chromic oxide with 4g of maize

Management of experimental birds

A total of 300, one-day-old, unsexed broiler chickens were raised on standard commercial starter diet. At day 20, selection for 216 male broilers chicks was made, each was tagged and weighed individually and were completely randomized before allotment to the six experimental diets in 3×2 factorial arrangement with 3 levels of P (1.40, 2.66 or 3.96 g/kg) arising from varying proportions of GNC and 2 levels of phytase (0 *FTU*/kg or 1000 *FTU*/kg). Six replicate cells were allotted to each dietary treatment and six birds per replicate cell. Allotment was done using an allotment programme of Kim and Lindemann, (2007). Birds were housed in metabolic cage containing 36 cells and during the 7 days feeding trial birds had access to clean water.

Data and sample collections

Feed intake was calculated on cell basis. Weighing at day 20 and 28 on cell basis to calculate average body weight changes. On day 28, birds were euthanized by carbon (IV) oxide for preceacal digesta collection from the two-third distal, end of the ileum using procedure of Rodehutscord *et al*., (2012). Preceacal digesta samples were milled and stored for analyses. Left tibiae from 4 birds were processed for determination of tibia ash.

Chemical analyses

Dry matter (DM) content for diets and preceacal digesta samples were analysed according to (AOAC International, 2005; method no: 930.15). Ashed samples for P and calcium were determined spectrophotometrically (FAO, 2011). Chromium (Cr) concentration in feed, preceacal digesta was determined by colorimetric method following digestion of ashed samples with nitric and perchloric acids with absorbance reading at 440ωm. Crude protein for GNC, diets and preceacal digesta samples were determined by total combustion method (AOAC International, 2005; method no: 968.06). Gross energy of GNC and diets was assayed using adiabatic bomb calorimetry standardized with benzoic acid.

Calculations

Apparent preceacal phosphorus digestibility (APPD) was calculated using index method according to Adeola, (2001)

$$\text{APPD (\%)} = 100 - \left(\left({}^{Cr_i}/_{Cr_o}\right) \times \left(\left({}^{P_o}/_{P_i}\right) \times 100\right)\right)$$

Cr_i is concentration of chromium in feed, Cr_o is chromium concentration in preceacal digesta, P_o represents phosphorus concentration from preceacal digesta and P_i denotes phosphorus intake from feed ingested. Analysed values were expressed as gramme per kg DM

Statistical analyses

Data were analysed using GLM procedure in SAS, (2004) and orthogonal polynomial contrasts were used to determine effects of P and phytase at 5% level of significance. Dietary P intake was expressed as P intake per kg DM intake. Preceacal digested P or total tract retained P (g/kg DM intake was regressed against P intake (g/kg DM) for (0 or 1000) *FTU* /kg, respectively, using the statistical linear regression model:

$$P_D = (TDP) \times P_I \pm EPPL$$

Where P_D represents preceacal digested P, EPPL represents intercept of regression curve. P_I represents P intake, slope estimates TPPD.

III. RESULTS

From tables 2 and 3, calculated CP values were higher than analysed values, while analysed calcium (Ca) values were higher than calculated values. Except for amount of P supplied at 210g of GNC, calculated P concentrations were higher than corresponding analysed values. Despite slight differences between analysed and calculated values, clear linear increase was obtained for CP, P and Ca.

Table 2. Analysed nutrient composition of GNC (as fed)

Composition	% (g/100g)
Dry matter	90.37
Crude protein	43.69
Ether extract	9.64
Calcium	0.09
Total phosphorus	0.36
Gross energy (kcal/kg)	4611.28

[1] each value is a mean of triplicate analysis

Table 3. Analysed nutrient composition of experimental diets

	Phytase, 0 FTU/kg			Phytase, 1000 FTU/kg		
	GNC 210g	GNC 420g	GNC 630g	GNC, 210g	GNC 420g	GNC 630g
Composition (%)						
Dry matter	93.00	91.58	92.68	92.62	93.09	90.37
Crude protein	19.25	23.10	36.75	17.15	25.90	35.35
Calcium	0.29	0.56	0.83	0.27	0.51	0.91
Total P (P)	0.17	0.22	0.29	0.16	0.27	0.29
Gross energy (kcal/kg)	4154	4126	3991	4137	4135	3887

[1] each value is a mean of triplicate analysis

Table 4. Selected growth performance indices and percentage tibiae ash of experimental birds

	Phytase (0 FTU/kg)			Phytase (1000 FTU/kg)				P-value						
											Without Phytase		With Phytase	
Measurements	GNC 210g	GNC 420g	GNC 630g	GNC 210g	GNC 420g	GNC 630g/	Pooled SEM[3]	Phytase	Phosphorus	Interaction	L^2	Q^2	L^2	Q^2
Feed intake (g/bird)	325.19	353.44	350.11	359.22	377.31	346.55	7.71	0.257	0.465	0.603	0.435	0.566	0.582	0.280
Dry matter intake(g/bird)	169.34	169.26	156.55	182.86	178.37	180.29	4.49	0.103	0.786	0.799	0.419	0.642	0.885	0.828
Body weight gain(g/bird)	59.57[a]	106.97[b]	139.08[c]	66.55[a]	105.39[b]	141.67[c]	6.49	0.746	<0.001	0.912	<0.001	0.601	<0.001	0.769
Feed : gain ratio	5.27[a]	3.38[b]	2.67[b]	5.15[a]	3.62[b]	2.47[b]	0.23	0.932	<0.001	0.792	<0.001	0.070	<0.001	0.406
Tibiae ash (%)	52.29[a]	47.66[ab]	44.95[b]	50.75[ab]	47.61[ab]	52.11[a]	0.95	0.309	0.198	0.125	0.025	0.902	0.557	0.946

[a b c] Means in a row with different superscripts are significantly different from each other (P<0.05) [1]Each value represents the mean of 6 replicates (6 birds/replicate) L^2 = Linear effect Q^2 = Quadratic effect (P=0.05) [3] Pooled standard error of mean, *P-Phosphorus

Table 5. Dietary P, P outputs and calculated phosphorus response criteria for experimental birds

Measurements	Phytase, 0 FTU/kg			Phytase, 1000 FTU/kg				P-value						
											Without Phytase		With Phytase	
	GNC 210g	GNC 420g	GNC 630g	GNC 210g	GNC 420g	GNC 630g	Pooled SEM[3]	Phytase	Phosphorus	interaction	L^2	Q^2	L^2	Q^2
P intake (g/kg DM)	1.82	2.35	3.16	1.71	2.94	3.26								
Preceacal P output (g/kg DMI)	0.38^{ac}	0.60^{a}	0.98^{b}	0.22^{c}	0.31^{cd}	0.57^{ad}	0.06	0.001	<0.001	0.435	0.003	0.523	0.001	0.743
Total tract P output	0.61^{a}	1.09^{b}	1.95^{c}	0.71^{ab}	0.73^{ab}	1.07^{b}	0.09	0.002	<0.001	0.006	<0.001	0.067	0.030	0.755
Preceacal Digested P	1.44^{a}	1.74^{c}	2.17^{c}	1.49^{b}	2.62^{d}	2.68^{d}	0.05	<0.001	<0.001	0.002	0.001	0.647	<0.001	<0.001
Total tract Retained P	1.21^{a}	1.25^{a}	1.20^{a}	0.37^{b}	2.20^{c}	2.18^{c}	0.12	0.006	<0.001	<0.001	0.281	0.344	<0.001	0.001
Apparent preceacal P digestibility (%)	79.14^{ab}	74.51^{ac}	68.91^{c}	87.27^{b}	89.42^{b}	82.49^{ab}	9.68	<0.001	0.061	0.636	0.093	0.917	0.354	0.223

$^{a\,b\,c}$ Means in a row with different superscripts are significantly different from each other (P<0.05) [1]Each value represents the mean of 6 replicates (6 birds/replicate)

L^2 = Linear effect: Q^2 = Quadratic effect (P=0.05) [3] Pooled standard error of mean, DM= dietary dry matter content, DMI = dry matter Intake, P-Phosphorus

Selected growth performance indices

As depicted in table 4, feed intake per bird showed no significant ($p>0.05$) response to either P or phytase supplementation levels, similar trend was observed for dry matter intake. Regardless of phytase, increasing dietary P intake influenced ($p<0.05$) average weight gain of birds. On comparison, average weight gain of birds fed with or without phytase for a specific graded level of GNC did not differ ($p>0.05$). Linear ($p<0.05$) increase in average weight gain of birds due to increasing P intake with corresponding better feed:gain ratios, were observed. Tibiae ash of birds showed no variation ($p>0.05$), values ranged between 47.61 to 52.06%.

Phosphorus digestibility and retention indices

From table 5, preceacal digesta P output of birds were significantly ($p<0.001$) affected by effects of P levels and phytase supplementation. As dietary P increased, lower preceacal digesta P outputs were observed for birds on phytase supplemented GNC diets in comparison with values obtained for birds fed GNC without phytase. Phytase addition accounted for 31.3% reduction in total tract P voided for birds on GNC diets with phytase. Preceacal digested and total tract retained P were not only affected ($p<0.05$) by main effects and interaction, but increased linearly ($p<0.05$) as GNC increased.

For GNC without phytase, (figure 1), TPPD coefficient (slope of regression curve) was 0.589 with corresponding EPPL value of 356.4mg of P/kg DMI. For phytase supplemented GNC (as shown in figure 2), TPPD coefficient was 0.793 and endogenous P loss of 170.1mg of P/kg DM intake

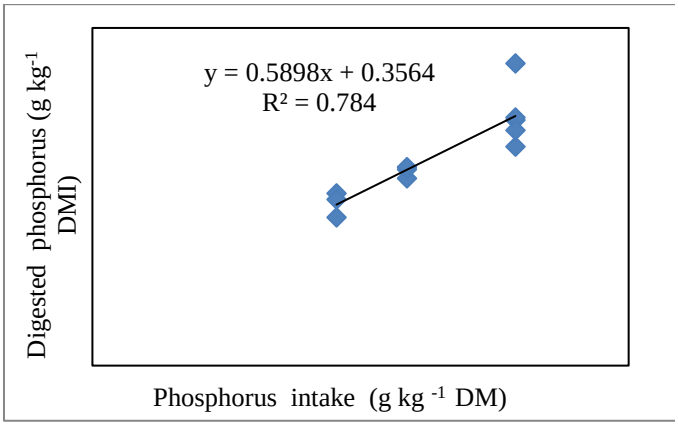

Fig. 1. Linear relationship between preceacal digested P and dietary P intake

Figure 1a. Regression curve for GNC without phytase supplementation

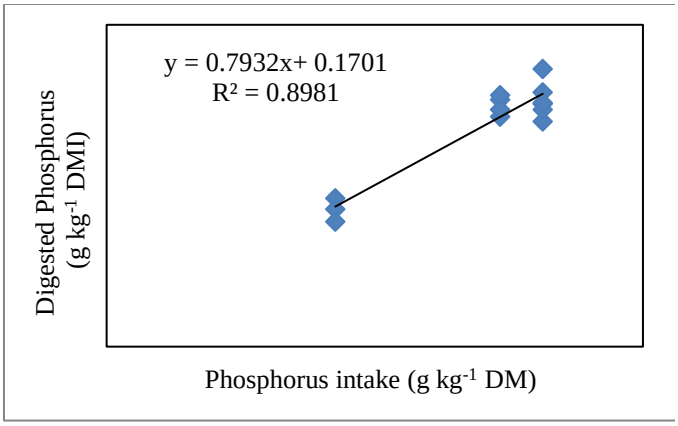

Figure 1b. Regression curve for phytase supplemented GNC

IV. DISCUSSION

Analysed dietary P in assay diets were close to calculated values except for P in diets containing 210g of GNC. Such differences between calculated and analysed values with regards to feeding P have been documented (Applegate and Angel, 2014). According to the authors, variations in ingredients nutrient profile, sampling and analytical procedures are usually associated with feeding P to broilers. It is important to note that despite the observed differences, graded proportions of GNC accounted for the increase in P across the diets, with latent increase in dietary protein supply to the birds even though research focus was not on dietary crude protein. From this study weight gain of birds increased as proportions of GNC increased. This observation indicates that at higher inclusions (430 or 645gm) of GNC, birds had more P available, for metabolic functions, skeletal development and body tissue growth. Lower body weight gain value was recorded for birds fed 210gm of GNC. Similar observation was also documented (Xue *et al*., 2016), who stated that low-crude protein diets decreased body weight gain and feed intake of experimental birds as well as compromising the absorption and utilisation of nutrients. Average weight gain values of birds fed 210g GNC mindless of phytase was 50-60% lower than those obtained for birds on 420 and 630g GNC. In addition, the dietary crude protein values at 210gm of GNC (17.5 or 19.2%) were lower than crude protein value range (22-24%) recommended for broiler starter chicks. Presumably, the extent to which birds fed 210gm of GNC were able to develop skeletal frame and body tissue growth seems affected by how much of P and protein are available in the diet. Such observation strongly indicates certain

relationship between P and protein. As described (NRC, 2012), there exist correlation between whole body content of N and P, stating that a reduction in one nutrient inadvertently affect deposition of the other. In support of this finding, studies (Mahan *et al.*, 1980; Carter and Cromwell, 1998) demonstrated that higher P is required to maximise nitrogen deposition in pigs. For Ferguson *et al*, (1998) feeding adequate or reduced CP and P to broiler chickens had significant interaction on growth performance. Regardless of phytase, body weight gain in birds was attributed to increasing P intake as GNC inclusion increased. Similar findings were also reported by Liu *et al*, (2013) and Mutucumarana *et al*, (2014). The better feed:gain ratio observed for birds at higher dietary P intake further affirms that increasing P intake closer to total P requirement (4.5 to 5g/kg diet) for broiler chickens, provides more P for growth and bone mineralization. Tibia ash, a sensitive indicator of bone mineralization was not influenced, despite phytase supplementation and increasing P intake. This finding is similar to those of Liu *et al.*, (2013) and Iyayi *et al.*, (2013) who reported no effect on tibia ash from their respective investigations. This observation could be due to the duration of feeding, which may not be adequate to induce significant changes in tibia ash. The simultaneous decrease in preceacal P concentration, as well as improved P digestibility coefficients for birds on phytase supplemented GNC further emphasizes the ability of phytase in hydrolyzing phytate-P complexes to release P even in non-conventional semi-purified diets. From the current study it was observed that APPD values obtained for birds fed 210gm of GNC, was not different from values observed for birds on 420gm of GNC, in contrast weight gain differed. This suggest that APPD might be independent of dietary crude protein but influenced by amount of P supplied in 210gm of GNC. This observation can probably be that hemostatic effect might play a role in helping the birds to retain P, even when dietary P intake was lower than dietary P intake at 430gm, birds on both inclusion levels of GNC recorded similar APPD values. Although the assay diets (cassava starch, wheat gluten diets) were different from the conventional cornstarch-casein based diets but phytase was able to improve P digestibility and retention of GNC in birds. This could probably be attributed to the favourable prevailing factors that influence phytase efficiency. According to Dersjant Li *et al*., (2014), the efficiency of feed phytases *in vivo* depends on interplay of phytase, dietary and animal- related factors. One key component of dietary related factors is Ca: P ratio. From the current study, estimate of Ca: P from analysed values (from 1.69:1 to 3.13:1) were higher than those obtained from calculated values (1.03:1 to 1.04:1) despite diets were formulated to ensure similar Ca: P across the diets. The high Ca:P could be attributed to the increasing proportion of limestone while trying to make adjustment for similar ratio across the diets. Though higher Ca: P were observed in the current study when compared with those of Liu *et al*, (2013) but these ratios seem not to have created an un-favourable condition for phytase activity based on the results obtained. From the results of this study, strong linear relationships were observed between preceacal digested P and dietary P intake, a basic requirement for the application of the regression technique in TPPD estimation. To achieve this, graded levels of test feedstuff assayed must triggers a gradual linear increase in levels of the nutrient under study. Other authors have reported strong linear relationship between; preceacal digesta P outputs and dietary P intake, total tract P outputs and dietary P intake (Fan *et al*., 2001; Akinmusire and Adeola, 2009). The relationship permits theoretical estimation of EPPL (intercept) and TPPD from regression curve. From regression curves obtained in this study, TPPD for phytase supplemented GNC was 79.32%, which was 4.48 points higher than estimate value obtained by (Iyayi *et al*., 2013), on feeding phytase supplemented peanut flour diets to broiler chicks. In most documented reports corn-starch and casein were mainly used in formulation of semi purified diets as against cassava-starch and wheat gluten used in the current study. It can be posited whether the composition of semi-purified assay diets for specific test feed ingredient have influence on estimate values for TPPD and EPPL, using regression technique remains to be investigated. Addition of phytase to GNC reduced EPPL by 52.27% at preceacal sampling section.

V. CONCLUSION

In a nutshell, supplementation of GNC with Natuphos at 1000(*FTU*)/kg improved TPPD of GNC with a subsequent reduction in endogenous phosphorus loss of 28 days old broiler chicken.

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