CRUDE PROTEIN DIGESTIBILITY IN BROILER CHICKENS FED RICE HUSK-BASED DIETS SUPPLEMENTED WITH PHYTASE

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ABSTRACT

A total of 288, one day-old Arbor acres broiler chicks (BC) fed broiler starter till day 20 were used to investigate the effect of phytase on the digestibility of crude protein. On day 21, the chicks were weighed and allotted to 6 treatments with 6 replicates of 8 birds each using a 3x2 factorial arrangement in a randomized complete block design. Between days 25 and 27 post-hatch, samples of fresh excreta were collected once daily, bulked and stored in the freezer at -4ºC. On day 28, the birds were euthanized with carbon (IV) oxide asphyxiation and dissected to obtain digesta from the distal two-third of the ileum. Six semi-purified diets with 150, 300 and 450g/kg rice husk (RH) and 0 or 1000 units of phytase (Natuphos) were formulated. Ileal crude protein (CP) of the birds was significantly (P< 0.05) reduced by feeding graded levels of RH, while interaction of phytase and CP had no significant effect on the excreta CP (P>0.05). Apparent CP digestibility and retention of the birds had no linear and quadratic (P>0.05) responses as the dietary supply of protein from RH increase. Increasing dietary concentration of protein intake from RH, addition of phytase and the interaction, influenced digested and retained CP significantly (P<0.05). Therefore, digestibility of crude protein by broiler chickens fed RH-based diets supplemented with phytase improved digested and retained crude protein in broiler chickens.

Keywords: Arbor acre, Digesta, Ilea, Excreta, semi purified diets

INTRODUCTION

Majority of the phosphorus (P) in grains and oilseed meals is in the form of phytate. Phytate is poorly utilized by poultry because they have limited intestinal phytase (Adeola and Sands, 2003). As a result, poultry diets are supplemented with inorganic P sources resulting in large amount of P in the diets which are subsequently passed into the environment through the feaces. The activity of intrinsic phytase in the diet and endogenous phytase in the digestive tract are not enough for the efficient hydrolysis of phytase (Applegate *et al.,* 2003). Commercial use of phytase enzyme has been found to solve this problem (Augspurger and Baker, 2004). The beneficial effect of phytase digestibility of CP and other nutrients have been reported by several authors. Supplementing poultry diets with crude protein based on feed formulation calculations may lead to its deficiency or excretion of excess dietary nitrogen into the environment. (Dersjant-li *et al.,* 2014). Dietary supplementation of CP with exogenous phytase is effective in improving CP digestion in poultry diets (Fan *et al.,* 2001; Papesova, 2000). Hence, continuous measurement is needed to ensure greater utilization of dietary CP so as to reduce their excretion into the environment (Mutucumarana and Ravindran, 2016).

Due to high cost of poultry feed in developing countries, poultry farmers use unconventional sources of protein as alternatives (Adesina *et al.,* 2022;, Animashahun *et al.,* 2018; Olaniran *et al.,* 2020). Rice bran is one of such protein sources with a potential for feeding poultry. Rice bran (RB) can be included in proportions ranging from 20% to 30% of pigs' diet, in the nursery phase (Gonzalez-Vega *et al*., 2014). Total phosphorus content of RB is high (1.67%) although about 80% is in the form of phytate, which is largely unavailable for poultry and swine (Rostagno *et al.,* 2011). Because of the possibility of including RB in piglets' diet and its high concentration of phytate, a study was conducted to determine the total digestibility of CP from RB, with or without the addition of phytase for broiler chickens.

MATERIAL AND METHODS

A total of 288, one-day-old Abor acres broiler chicks were fed a starter diet till day 20. Vaccination and other routine management practices were carried out. On day 21, the chicks were individually weighed and distributed into 36 cages in a randomized complete block design with 6 replicate cages per treatment and 8 birds per cage using Experimental Animal Allotment Programme (EAAP). Six semi-purified diets of 3 levels (150, 300 and 450g/kg) of CP, without and with phytase (1000FTU/kg, Natuphos, BASF Germany) respectively, obtained by the gradual replacement of cassava starch with rice husk were formulated (Table 1). Titanium dioxide was added as an indigestible marker at the rate of 5g/kg of diet. On days 23, 24 and 25, fresh excreta samples were collected from trays placed beneath each cage at 24-hourly intervals and dried at 55^{o}C using a force draught oven. The experiment lasted 28 days. On day 26, the birds were weighed and slaughtered and digesta from the last 2/3rd of the ileum (Rodehutscord *et a*l., 2012) was collected, and pooled according to cage, frozen and dried. Samples of RH, experimental diets, digesta and excreta were analysed for CP according to the methods of AOAC (2000). Titanium concentration was determined using a colorimetric assay (Short *et al.,* 1996).

Table 1. Gross composition of experimental diets (g/kg) (as-fed basis)

	0 FTU/Kg (Phytase)			1000FTU/Kg (Phytase)		
Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Rice Husk (RH)	150.00	300.00	450.00	150.00	300.00	450.00
Cassava Starch	464.50	316.25	168.00	454.50	306.25	158.00
Wheat gluten	220.00	220.00	220.00	220.00	220.00	220.00
Soya oil	20.00	16.25	12.50	20.00	16.25	12.50
Dextrose	102.25	102.25	102.25	102.25	102.25	102.25
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	11.25	13.25	15.25	11.25	13.25	15.25
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 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
Analysed Nutrients						
Gross energy (kcal/kg)	3458.06	3355.23	3252.40	3423.16	3320.33	3217.50
CP (g/kg)	18.54	20.63	24.5	19.11	20.73	22.45
Ca (g/kg)	0.53	0.58	0.62	0.34	0.45	0.53
Total P (g/Kg)	0.21	0.24	0.30	0.22	0.26	0.32
Dry matter	97.35	97.01	96.46	97.24	97.6	96.54

[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

Calculations and Statistical Analyses

Data were analysed using the GLM procedure of SAS (SAS Institute, 1999). Orthogonal polynomial contrast was used to determine linear and quadratic effects of CP level, phytase and their interaction on all response criteria with α level of 0.05 considered as significant.

Total CP $_{output}$ $(CP_O) = CP_i \times Ti_{diet}/Ti_{excreta\ or\ digesta}$

AND, % $= 100 - [(Ti_{diet} / Ti_{digesta\ or\ excreta}) \times (N_{digesta\ or\ excreta}/N_{diet}) \times 100]$

Where, CP_i = Crude Protein intake, Ti_{diet} =Titanium in diet, $Ti_{excreta\ or\ digesta}$ = Titanium in excreta or digesta, AND = Apparent nutrient digestibility, $N_{digesta\ or\ excreta}/N_{diet}$ = Nitrogen in excreta or digesta.

RESULTS

Table 1 shows the gross composition of experimental diets fed to the broiler chickens. Body weight gain was not (P>0.05) affected as a result of feeding graded levels of RH (Table 2). Presented in Table 3 are results of crude protein outputs, crude protein digestibility and retention of experimental birds. Crude protein contents of diets increased as dietary proportion of RH increased. Ileal crude protein outputs of the birds were not affected (P>0.05) as a result of feeding graded level of RH supplemented with phytase and its interaction (CP x phytase). For birds on RH diets without phytase, ileal crude protein outputs values were; 2.72, 3.67 and 4.52g/kg DM intake. On adding phytase, the observed ileal crude protein outputs were; 3.10, 2.69 and 3.89g/kg DM intake. On the other hand, crude protein voided was not significantly (P>0.05) affected by increase in dietary CP intake and phytase supplementation. There was no linear and quadratic (P>0.05) response in the excreta crude protein of birds fed RH diets without and with phytase supplementation as the inclusion of RH increased in the diets. The excreta crude protein values for birds with or without phytase were; 10.11, 10.93 and 7.16g/kg DM intake compared to 12.03, 8.05 and 9.48g/kg DM intake respectively. Increasing dietary concentration of CP intake from RH, addition of phytase and the interaction, CP x phytase influenced digested and retained crude protein significantly (P<0.05). Linear and quadratic (P<0.05) responses were also observed in the digested and retained crude protein. Birds fed diets containing 450g RH/kg diet, with or without phytase had high crude protein values of 249.00 and 228.69g/kg DM intake respectively. Apparent crude protein digestibility values for birds ranged from 98.22 to 98.57% for birds on diets without phytase, a range of 98.33 to 98.73% were observed for birds on phytase supplemented RH-based diets. Apparent crude protein retention values ranged from 93.68 to 96.27% for birds that had diets without phytase compared to range of 94.85 to 96.92% for birds on diets with supplemental phytase. Birds had higher crude protein digestibility values than their retention values indicating higher ileal digestion than retention. Apparent crude protein digestibility and retention of the birds showed no linear and quadratic (P>0.05) responses as the dietary supply of protein from RH increase.

Table 2. Selected growth performance indices and percentage tibiae bone ash of 28-day-old broilers1 fed rice husk-based diets

	Without Phytase g/kg of diet			With Phytase g/kg of diet				P-value						
											Without Phytase		With Phytase	
Items (g/b)	RH 150	RH 300	RH 450	RH 150	RH 300	RH 450	Pooled SEM	Phyt	CP	Phy x CP	L^2	Q^2	L^2	Q^2
FI	157.97	173.80	175.4	181.3	188.13	199.9	6.54	0.130	0.543	0.944	0.460	0.725	0.43	0.91
IWt	371.93	373.36	377.8	383.5	376.03	388.5	6.14	0.141	0.723	0.874	0.543	0.856	0.58	0.89
FW	485.96	500.06	501.9	498.9	505.16	512.5	7. 52	0.174	0.911	0.994	0.652	0.931	0.76	0.86
BWG	114.03	126.70	124.2	115.4	129.13	124.0	2.38	0.799	0.008	0.975	0.182	0.244	0.36	0.25

[a b c] Means in a row with different superscripts are significantly different from each other (P<0.05), Each value represents the mean of 6 replicates (8 birds/replicate) L^2 = Linear effect, Q^2 = Quadratic effect (P=0.05) [3] Pooled standard error of mean, ,*CP-Crude Protein, FI=Feed intake, BWG=Body weight gain, IWt=Initial weight, FW= Final Weight

Table 3. Crude protein intake, CP outputs and calculated response criteria of 28-day-old broilers fed Rice Husk based Diets

	Without Phytase g/kg of diet			With Phytase g/kg of diet				P-value						
											Without Phytase		With Phytase	
Items	**RH 150**	**RH 300**	**RH 450**	**RH 150**	**RH 300**	**RH 450**	**PSEM**	**Phyt**	**CP**	**Phy x CP**	**L^2**	**Q^2**	**L^2**	**Q^2**
CPg/kg	190.4	212.6	253.9	196.5	212.4	232.5	….	….	…	…	…	…	…	…
IC P (DMI)	2.72^{a}	3.67^{ac}	4.52^{c}	3.1^{ac}	2.69^{a}	3.89^{ac}	0.23	0.34	0.04	0.40	0.02	0.94	0.31	0.24
DCP (DMI)	187.7^{a}	209^{b}	249^{C}	193^{d}	210^{b}	229^{e}	3.54	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.06
ACPD (%)	98.5	98.2	98.2	98.4	98.7	98.3	0.09	0.45	0.50	0.39	0.24	0.63	0.78	0.23
ECP (DMI)	12.03	8.05	9.48	10.11	10.93	7.16	0.70	0.75	0.28	0.24	0.33	0.23	0.20	0.23
RCP (DMI)	178.4^{a}	205^{b}	245^{c}	186.4^{a}	201^{b}	225^{c}	3.85	0.002	<0.0001	<0.0001	<0.01	0.007	<0.01	0.04
ACPR (%)	93.7	96.2	96.3	94.9	94.9	96.9	0.36	0.82	0.03	0.28	0.05	0.25	0.08	0.30

$^{a\,b\,c}$ 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 (8birds/replicate) L^2 = Linear effect: Q^2 = Quadratic effect (P=0.05). PSEM=Pooled standard error of mean, Ileal Crude Protein=(ICP), Apparent CP digestibility= ACPD, Digested CP=DCP, Excreta CP, Excreta CP=ECP, DM= Dietary dry matter content, ACP Retention: ACPR, DMI = dry matter intake, Retained CP=RCP

DISCUSSION

The level of rice husk inclusion or its interaction with phytase supplementation did not affect the apparent CP digestibility and retention of birds. This suggests that RH can be included up to 45% in the diets of broilers. The results obtained suggests that supplementation of rice husk diets with phytase is effective in improving the bioavailability of CP. This report supports the work of Iyayi (2013), this author used cowpea as its test ingredient to improve CP digestibility with the supplementation of phytase. As diets were supplemented with phytase, digestibilities and retention of CP (Table 4) were improved. The release of P from phytate complex is accompanied by the release of other nutrients. The effect of this is improved digestibility of CP in the diets and the release of phytate P in RH. Graded levels of RH did achieve the aim of increasing dietary CP intake. As dietary proportions of the test ingredients increased across the diets, nitrogen excretion increased (excreta CP). Some authors have asserted that interaction of dietary CP and P and their subsequent metabolic utilization are lacking. Lending credence to this assertion is the report of Adeola and Sands (2004) who reported that no significant interactions between CP and non-protein nitrogen (NPP) were detected for any of the growth performance criteria investigated in broiler chickens. In contrast, studies conducted in pigs (Cromwell *et al.,* 1993 and Ferguson *et al.,* 1998) have shown that concentration of protein in the diet is connected to P requirement and that higher concentrations of P are required to maximize nitrogen deposition in pigs. Ferguson *et al.* (1998) concluded that feeding adequate or reduced CP and P concentrations to broiler chicks resulted in a significant CP and P interactions on growth performance. These conclusions in a nutshell, strongly suggests that adequate dietary supply of P from RH will only be possible at higher inclusion level of the test ingredient, with resultant increase in CP. From this study, the observed increase in nitrogen excretion across the sequential diets strongly indicates that increases in nutrient output at the highest concentration of dietary CP intake suggests that either absorption is reduced, the efficiency of metabolic utilization is lower or both mechanisms may be responsible. Apart from the graded level of the assayed ingredients, wheat gluten served as the baseline protein in the sequential diets. Also, the dietary manipulations across the study were done to ensure that crude protein of the diets were close to and above the CP requirement (24%) for starter broiler chicken. Adeola and Sands (2003) reported that while supplemental microbial phytase has an improvement in utilization of plant-derived phytin, review findings in literature (Iyayi, 2013) suggests that it may not affect CP digestibility. Nevertheless, response of CP to phytase supplementation can be due to complexes present in feedstuffs, *de novo* formation of protein complexes during intestinal transient of the animal, *de novo* formation of phytin-free amino acid complexes during gastrointestinal passage in the animal and complexes involving phytin and proteolytic enzymes (Selle *et al.,* 2000). Hence in this study, there was no response of apparent CP digestibility to phytase due to the reasons of these authors. Apparent ileal CP digestibility for birds fed RH independent of phytase supplementation ranged from 83.46 to 94.43 and 98.22 to 98.57% respectively. Simultaneously, apparent CP retention values ranged from 76.35 to 88.31 and 93.68 to 96.27%. Higher apparent ileal CP digestibility compared to total tract retention values implies that the experimental birds were able to digest the protein from the assayed test ingredient. Body weight gain was not affected by feeding experimental diets; this could be as a result of the short period of the experiment. Results from this study suggest that Natuphos phytase which is a 3-enzyme phytase was effective in improving CP digestibility.

CONCLUSION AND RECOMMENDATIONS

It can be concluded from the study that supplementation of RH up to 450g/kg with 1000 units of phytase improved the digestibility of CP without any detrimental effect. Farmers are encouraged to formulate broiler diets with RH supplemented with phytase to achieve optimum absorption of appropriate nutrients and to maximize nitrogen deposition.

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