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Performance, blood profile and microbial characteristics of broiler chicken fed phytase supplemented diets

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Abstract

Phytate is an anti-nutritional factor found in grains which limits the availability of essential minerals in monogastric nutrition as they lack enzymes to break it down. Its effect on performance, nutrient retention and availability of many nutrients including the macro elements and trace minerals have been documented in many studies in different animal species with conflicting results. Thus, this study was conducted to evaluate the performance, blood metabolites and microbial population in broiler chicks fed graded levels of phytase enzyme at 0, 250, 500, 750 and 1000FTU/kg of the experimental diet in a 56-day feeding trial. One hundred and ninety-five, one-day-old Arbor acre chicks were randomly allotted into five dietary treatments in a completely randomized design and the treatment had three replicates with 13 birds each. Data were collected on performance characteristics weekly while on day 56, blood was collected at the jugular vein of two birds per replicate for haematological and serum biochemical parameters. Consequently, two birds per replicate were also sacrificed and excised for jejunum and ileum microflora assay. Result for the performance characteristics showed no significant (P>0.05) differences in final bodyweight, daily weight gain, feed intake and feed conversion ratio. Initial body weight ranged from 36.08±0.78-40.13±3.57g/bird, final body weight ranged from 2192.86±28.87-2466.81±117.40g/bird and the Feed conversion ratio also ranged from 2.78-3.21. Also, there were no significant (P>0.05) differences observed in the haematological and serum biochemical parameters but numerical differences exist. Packed cell volume had values between 21.33-26.00% while haemoglobin also had values ranging from 7.11-8.67g/100mL. However, significant (P<0.05) differences exist in the ileum while no significant (P>0.05) differences were found in jejunum tissues. For Aspergillus flavus, 59.4±5.1cfu/mL was significantly (p<0.05) higher than 8.4±3.0cfu/mL for the ileum tissue while the jejunum tissue had values ranging from 4.2±1.80-16.8±6.00mL which were not significantly (P>0.05) different. In addition, significant (p<0.05) differences were observed for the ileum and jejunum tissue for Escherichia coli. The ileum tissue had significant values that ranged from 3.3±0.9-24.6±4.8mL while the jejunum also had significant values that ranged from 4.5±0.3-27.9±5.7mL. In conclusion, the use of microbial phytase had no influence on the performance of broilers and blood metabolite but has effect on the microflora of broiler chicken.

Keywords: Phytate, Performance, Blood metabolite, Microbial load, Broiler chicken

Introduction

Feed has great economic importance on poultry production, as it contributes about 60-70% of total cost of production (Coon, 2002). Bioavailability of nutrients and its utilization by poultry cannot be overemphasized. Therefore, a great deal of attention has recently been received from nutritionists for proper utilization of nutrients and the use of feed additives such as enzymes, probiotics, prebiotics, among others for improved growth in poultry. Phytate is the major form of phosphorus (P) abundantly found in cereal grains, beans and oilseed meals used mostly in monogastric diets. Poultry birds are unable to utilize this source of phosphorus due to lack of endogenous phytase enzyme.

Phytate is not only an unavailable source of phosphorus (P) for broilers but also acts as an anti-nutrient, reducing protein and mineral absorption, increasing endogenous losses and thus reducing broiler performance. It has been reported that adverse effects of phytate can be decreased by the supplementation of phytase in the diet. Guo *et al*. (2009) found better performance and bone characteristics in broilers fed diets supplemented with sodium gluconate and phytase. Vinil *et al*. (2000) also found a reduction in feed cost in soy-wheat bran diets supplemented with enzyme. However, Cabahug *et al*. (1999) averred that the efficacy of phytase depends upon the enzyme application level and the amount of phytate present in the diet, Ca: P ratio, genotype and age of birds. Dietary phytase inclusion has been reported to have beneficial effects on the growth performance, feed efficiency, protein/amino acid digestibility, energy utilization, mineral retention and bone growth of broilers due to the direct hydrolytic effects on phytate (Cabahug *et al.*, 1999; Cowieson *et al*., 2006). Its effect on performance, nutrient retention and availability of many nutrients including the macro elements and trace minerals have been documented in many studies in broiler chicken with conflicting results. Therefore, this study was designed to evaluate the performance, blood profile and microbial population of broiler chicken fed diets supplemented with graded levels of phytase.

Materials and methods

The experiment was carried out at the Poultry Unit, Teaching and Research Farm, University of Ibadan, Nigeria. The feed ingredients and phytase used were purchased from a commercial farm store in Ibadan, Oyo state, Nigeria.

Experimental birds and diets

One hundred and ninety-five (195) one-day old Arbor Acre broiler chicks were randomly allotted into five dietary treatments with three replicates of thirteen birds each. A broiler starter diet containing 23.23% crude protein (CP) and 3062.11 Kcal/kgME was fed to the birds for four weeks. At the finishers phase, diets containing 20.65% CP and 2983.91 Kcal/kgME was used for the remaining four weeks. The levels of phytase supplementation were 0, 250, 500, 750 and 1000 FTU/kg diet respectively as shown in Tables 1 and 2.

Table 1: Gross composition (g/100gDM) of starter diets (supplemented with graded levels of phytase) fed to broiler chicks

	Phytase Inclusion level				
Ingredient	control	250FTU	500FTU	750FTU	1000FTU
Maize	51.00	51.00	51.00	51.00	51.00
Fish meal(72%)	3.00	3.00	3.00	3.00	3.00
Soyabean meal	36.00	36.00	36.00	36.00	36.00
Wheat offal	4.80	4.80	4.80	4.80	4.80
Di-calcium Phosphate	1.50	1.50	1.50	1.50	1.50
Oystershell	1.00	1.00	1.00	1.00	1.00
Table salt	0.25	0.25	0.25	0.25	0.25
Palm oil	2.00	2.00	2.00	2.00	2.00
Methionine	0.15	0.15	0.15	0.15	0.15
Lysine	0.05	0.05	0.05	0.05	0.05
Vitamin-mineral Premix	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100

Calculated nutrients: crude protein -20.65%,crude fibre -4.18%, energy -2983.91(Kcal/kgME),ether extract -5.55%, calcium - 1.08%,phosphorus-0.33% .Vitamin-mineral Premix composition - Calcium- 27%,starch-0.04%, crude fibre -0.03%, Vitamin A (E672) - 4000000 IU, Vitamin D3 (E671) - 800000 IU, Vitamin E (all -rac-α-tocopheryl acetate) (3a700) - 12000 IU, Vitamin B1 (thiamine mononitrate)- 800 mg, Vitamin B2 (riboflavin) - 2000 mg, Vitamin B6 (pyridoxine hydrochloride) (3a831) - 1600 mg, Vitamin B12 (cyanocobalamin)- 8000 µg, Vitamin K3- 800 mg, Pantothenic acid- 4000 mg, Niacin- 16000 mg, Biotin- 60000 µg, Folic acid- 400 mg, Choline chloride- 60000 mg, iron- 16000 mg, Iodine- 400 mg, Copper- 4000 mg, Manganese- 32000 mg, Zinc- 24000 mg, Selenium- 60 mg

Table 2: Gross composition (g/100gDM) of finisher diet (supplemented with graded level of phytase) fed to broiler chickens

	Phytase Inclusion level				
Ingredient	control	250FTU	500FTU	750FTU	1000FTU
Maize	50.00	50.00	50.00	50.00	50.00
Fish meal(72%)	0.00	0.00	0.00	0.00	0.00
Soyabean meal	32.00	32.00	32.00	32.00	32.00
Wheat offal	12.80	12.80	12.80	12.80	12.80
Di-calcium Phosphate	1.50	1.50	1.50	1.50	1.50
Oystershell	1.00	1.00	1.00	1.00	1.00
Table salt	0.25	0.25	0.25	0.25	0.25
Palm oil	2.00	2.00	2.00	2.00	2.00
Methionine	0.15	0.15	0.15	0.15	0.15
Lysine	0.05	0.05	0.05	0.05	0.05
Vitamin-mineral Premix	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100

Calculated nutrients: crude protein -20.65%,crude fibre -4.18%, energy -2983.91(Kcal/kgME),ether extract -5.55%, calcium - 1.08%,phosphorus-0.33% .Vitamin-mineral Premix composition - Calcium- 27%,starch-0.04%, crude fibre -0.03%, Vitamin A (E672) - 4000000 IU, Vitamin D3 (E671) - 800000 IU, Vitamin E (all -rac-α-tocopheryl acetate) (3 a700)- 12000 IU, Vitamin B1 (thiamine mononitrate)- 800 mg, Vitamin B2 (riboflavin) - 2000 mg, Vitamin B6 (pyridoxine hydrochloride) (3a831) - 1600 mg, Vitamin B12 (cyanocobalamin)- 8000 µg, Vitamin K3- 800 mg, Pantothenic acid- 4000 mg, Niacin- 16000 mg, Biotin- 60000 µg, Folic acid- 400 mg, Choline chloride- 60000 mg, iron- 16000 mg, Iodine- 400 mg, Copper- 4000 mg, Manganese- 32000 mg, Zinc- 24000 mg, Selenium- 60 mg

Data collection

Feed supplied and leftover were weighed on weekly basis to calculate the feed intake. Birds were also weighed on weekly basis. At the end of the experiment, two birds per replicate were selected and bled through the jugular vein using hypodermic needle with syringe. Blood was drained into 2 different bottles for haematological indices and serum biochemical metabolites. The blood samples for haematological parameters were collected into a bottle which has been pre-treated with EDTA (Ethylene Diamine Tetra Acetic Acid). Blood samples for serum biochemical indices were collected into another bottle without EDTA. Consequently, two birds per replicate were also sacrificed, excised and 5cm of the jejunum and ileums were harvested for microflora assay. Total bacterial count of the representative sample from caeca of birds was carried out on nutrient agar by plate count method. Serial dilutions were made and 100 µL of the final dilution was spread uniformly on nutrient agar plates which were incubated at 37°C for 48 hrs. Different colonies obtained were stained using Gram's stain and subsequently count was done for different bacteria types and estimated in $\log_{10}$ CFU/mL according to the method described by Wynn *et al*. (2013).

Experimental design

The experimental birds were allotted to treatment in a completely randomized design.

Statistical analysis

Data collected were subjected to one-way analysis of variance using SAS (2009) package and means were separated using Duncan multiple range test of the same software.

Results and discussion

Table 3: Performance characteristics of broiler finisher fed diets supplemented graded level of phytase

Parameter	Control	250FTU	500FTU	750FTU	1000FTU	P value
Final body weight (g/birdx10^2)	22.54±83.02	22.79±189.30	23.62±74.00	24.67±117.40	21.92±28.87	0.5000
Average Daily weight gain (g/bird)	39.54±1.48	39.92±3.34	41.48±1.33	43.33±2.1	38.45±0.51	0.495
Feed intake (g/bird/day)	126.53±7.86	115.36±2.93	120.11±1.03	119..96±0.79	122.42±1.27	0.3990
Feed conversion ratio	3.21	2.92	2.90	2.78	3.19	0.287

[abc:] **Mean along the row with the same superscript are not significantly (P>0.05) different from each other**

The result of performance characteristics of broilers fed enzyme supplemented diet are presented on Table 3. There were no significant ($p>0.05$) differences in all parameters measured although numerical differences exist. Final body weight ranged from 2192.86±28.87-2466.81±117.40g/bird and the Feed conversion ratio also ranged from 2.78-3.21. Lima *et al*. (2002) fed diets supplemented with two levels of phytase to broilers chickens and verified that there were no significant differences in the performance of the birds. Assuena *et al.* (2007) also did not observe benefit from supplementation with phytase in diets for broilers. Manafi *et al*. (2001) suggested that broilers finishers fed enzyme supplemented diets have increased feed intake due to increased nutrient digestibility which is averred to the results obtained from this study. The feed intake of control diet (no phytase supplementation) in this study was found to be the highest (126.53g/bird). Knap *et al*., (1996) suggested that body weight gain and feed conversion in broilers from 1 to 42 days were improved in broilers fed corn and soybean meal diets containing α-galactosidase (enzyme) over birds fed identical diets without the enzyme. Improvements in feed conversion may be attributed to improved energy availability in soybean meal caused by the enzyme supplementation which is a general observation with the result of this study. The birds on phytase supplementation had better feed conversion ratios (2.78-2.92) compared to the control with a value of 3.21. Thus, the utilization of phytase in diets of minimum cost can be an alternative for obtaining better profitability in broiler production. Treatments with phytase supplementation had better conversion ratio indicating its importance in broilers production.

Table 4: Haematological parameters of broilers finisher fed diets supplemented graded levels of phytase

Parameters	Control	250FTU	500FTU	750FTU	1000FTU	SEM	P value
Monocytes (cfu)	2.00	2.67	2.67	3.00	3.33	0.33	0.5897
Eosinophilus (10^9/µl)	3,00	3.33	3.00	2.00	3.67	0.39	0.6690
Basophilus (10^9/µl)	1.67	0.00	1.33	1.67	0.33	0.38	0.4083
Plateelet (10^9/µl)	1.9	1.73	20.63	1.70	1.82	0.09	0.9789
Erythrocyte Sedimentation Rate (mm/h)	0.40	0.43	0.33	0.30	0.40	0.10	0.3592
Packed cell vol (%)	26.00	25.00	22.67	24.67	21.33	0.69	0.6736
Haemoglobin (g/100ml)	8.67	8.33	7.55	8.22	7.11	0.40	0.6732
Red blood cell (10^6/ul)	4.14	3.84	4.20	4.02	3.84	0.28	0.9555
White blood cell (10^3/ul)	16.82	17.99	19.27	20.88	20.47	0.62	0.5852
Neutrophile (g/dl)	30.33	30.00	32.33	35.00	36.33	0.88	0.7517
Lymphocyte (g/dl)	63.00	65.00	60.67	58.67	56.67	0.92	0.6875

[abc]: Mean along the row with the same superscript are not significantly (P>0.05) different from each other

Table 5: Serum biochemistry of finisher birds fed diets supplemented with graded level of phytase

Parameters	Control	250FTU	500FTU	750FTU	1000FTU	SEM	P value
TP (g/dl)	2.19	2.28	2.23	2.52	2.22	0.20	0.7810
ALB (g/dl)	1.49	1.47	1.36	1.53	1.35	0.46	0.7861
AST (U.I/I)	145.71	159.50	178.52	123.55	161.77	1.96	0.4494
ALT(U.I/I)	6.03	8.66	6.29	4.37	7.36	0.69	0.7959
GLO (g/dl)	0.70	0.81	0.87	0.99	0.87	0.16	0.6113

[abc]: Mean along the row with the same superscript are not significantly (P>0.05) different from each other

Table 4 shows the haematological parameters of broilers chicken fed graded level of phytase enzyme. Blood hematological parameters serve as indicators of the physiological state of birds (Castagliulo *et al*., 1996; Sarker *et al*., 1996; Chowdhury *et al*., 2005). Haematological indices are an index and a reflection of the effects of dietary treatments on the animals in terms of type, quality and amount of the feed ingested and were available for the animal to meet its physiological, biochemical and metabolic necessities (Ewuola *et al*., 2008). The results observed from this study were not significantly influenced by phytase supplementation. Packed cell volume had values between 21.33-26.00% while haemoglobin also had values ranging from 7.11-8.67g/100ml. Chuba *et al*. (2014) revealed no significant difference in haematological parameter of broilers fed probiotics and commercial enzyme. Similarly, the result of this study is in agreement with report of Shehab *et al*. (2012) that phytase inclusion in Japanese quail diet does not adversely affect the haematological constituents of the birds. The result of this study indicated that all the haematological parameters measured fell within the normal (reference range) for healthy chickens, which suggested that the diets were well tolerated by the experimental animals. The results of the Serum biochemistry of broilers fed graded levels of phytase enzyme are presented in Table 5. A serum biochemistry profile is a blood test that provides an overview of many of the body's functions of the birds. Total protein have values ranging from 2.19-2.52g/dL, albumin values ranged from 1.35-.53g/dL and globulin have values that ranged from 0.7-0.99g/dL. The results of this study indicate significant ($p>0.05$) differences were not observed in all the parameters measured. This is in consonance with the findings of Owosibo *et al*. (2013) where no significant ($P>0.05$) differences were observed in all the serum biochemical indices examined except for the cholesterol in marshal broilers chicks. However, Rahman *et al*. (2013) found significant differences ($p.<0.05$) in Aspartate aminotransaminase(ALT) and alanine aminotransaminase (AST) of broilers fed probiotics. Thus, the inclusion of phytase did not alter the blood metabolite profile of broilers in this study.

Table 6: Microbial count of ileum and jejunum of broiler finisher fed diet supplemented with exogenous enzyme (CFU×10^5/ml)

Bacteria(cfu/ml)	Tissue	Control	250FTU	500FTU	750FTU	1000FTU	P value
Escherichia coli	ileum	$24.6±4.8^{c}$	$15.0±4.2^{bc}$	$20.7±0.3^{bc}$	$3.3±0.9^{a}$	$9.9±2.1^{ab}$	0.022
	jejunum	$14.1±3.3^{ab}$	$27.9±5.7^{b}$	$11.7±6.9^{ab}$	$4.5±0.3^{a}$	$10.5±3.3^{a}$	0.093
Aspergillus flavus	Ileum	$12.9±0.3^{a}$	$3.6±1.8^{a}$	$27.3±2.7^{b}$	$8.4±3.0^{a}$	$59.4±5.1^{c}$	0.000
	jejunum	$4.2±1.80^{a}$	$13.2±1.80^{a}$	$4.8±2.4^{a}$	$9.6±3.6^{a}$	$16.8±6.00^{a}$	0.183

^{abc}: Mean± standard error along the row with the same superscript are not significantly (P>0.05) different from each other

Table 6 represents the results of microbial load of birds in experimental diets fed phytase enzyme. The GIT of an animal can be considered a micro ecosystem that provides a specific niche for intestinal bacterial communities to reside, and in turn the bacteria act in an intraorganismal mutualism with the host animal for optimal benefit of the two (Xu *et al*., 2003; Apajalahti, 2005). Significant ($P<0.05$) differences were observed in the ileum tissue while no significant ($P>0.05$) difference were observed in jejunum tissues for *Aspergillus flavus*. 59.4±5.1cfu/mL was significantly ($p<0.05$) higher than 8.4±3.0cfu/mL for the ileum tissue while

the jejunum tissue had values ranging from 4.2±1.80-16.8±6.00 which were not significantly different (P>0.05). *I*n addition, significant differences were observed for the ileum and jejunum tissue for Escherichia coli. The ileum tissue had significant values that ranged from 3.3±0.9-24.6±4.8 while the jejunum also had significant values that ranged from 4.5±0.3-27.9±5.7. For the ileum, the treatment with 500FTU phytase supplemented diet had the highest microbial counts which suppose higher microbial action compared to other treatments. For the jejunum, 500FTU phytase supplemented diet had the highest microbial count (33.67×105/mL) which is also implicated for higher microbial activity. The result obtained in this result is in agreement with the findings of Hassan *et al*., (2014) which showed significant differences($p<0.01$) in the bacterial count of the intestine of birds fed different direct-fed microbials.

Conclusion

The study showed that the use of microbial phytase had no significant influence on the performance of broilers and blood metabolite but had effect on the microflora of broiler chicken.

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