Asian Journal of Advances in Agricultural Research

2(4): 1-7, 2017; Article no.AJAAR.36468
ISSN: 2456-8864

Haematological Parameters, Serum Metabolites and Enzyme Activities of Broiler Chicken Fed with or without Phytase

O. T. Daramola[1*]

[1]Department of Agricultural Technology, Federal Polytechnic, P.M.B 5351, Ado-Ekiti, Ekiti –State, Nigeria.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/AJAAR/2017/36468
Editor(s):
(1) Daniele De Wrachien, Department of Agricultural and Environmental Sciences,The State University of Milan, Italy.
Reviewers:
(1) G. K. Sivaraman, ICAR- CIFT, India.
(2) Zar Chi Thent, Universiti Teknologi MARA, Malaysia.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/21198

Original Research Article

Received 29th August 2017
Accepted 18th September 2017
Published 2nd October 2017

ABSTRACT

Aim: The experiment was conducted to investigate the effect of phytase on haematological indices, serum metabolites and enzyme activities of broiler chickens fed diets with or without phytase supplementation.
Study Design: The experiment employed a complete randomized design; all data generated were subjected to analysis of variance, P=0.05.
Place and Duration of Study: The study was carried out at the Teaching and Research Farm of the Ekiti State University, Ado-Ekiti, Nigeria, between February and April, 2011.
Methodology: Two hundred and forty unsexed day-old Anak 2000 strain broiler chicken were used in a 56-day feeding trial. The birds were allotted to 5 treatments with 4 replicates per treatment of 12 birds per replicate. Diet 1 was the reference diet with no phytase supplementation but with both plant and animal protein sources. Diets 2 and 3 were duplicate diets with enzyme supplementation only in diet 3. Diets 4 and 5 were also duplicate diets with enzyme supplementation only in diet 5. Diets 2 and 3 had groundnut cake as the major plant protein ingredient while diets 4 and 5 had soybean cake as the major protein ingredient. They were fed *ad-libitum.* Haematological parameters, serum metabolites and enzyme activities were determined.
Results: Significant (P<0.05) increase was obtained for mean corpuscular haemoglobin

**Corresponding author: Email: olajumoke.daramola2016@gmail.com;*

concentration (MCHC) of birds on diet 3 (30.04±2.41%) and diet 5 (29.74±2.30%), the eosinophils of birds on reference diet (4.80±1.29%) aspartate amino transferase (AST) of birds on diet 3 (103.50±1.01 mmol/L) and diet 5 (105.00±0.01 mmol/L). However, significant (P<0.05) decrease was recorded for alkaline phosphate (ALP) of birds on phytase supplementation, diet 3 (116.42±3.73 mmol/L) and diet 5 (118.80±1.51 mmol/L).

Conclusion: The parameters evaluated in this study did not indicate any changes that would suggest that phytase supplementation in broiler diets affected the health status of broiler chicken.

Keywords: Haemotology; serum biochemistry; enzyme activities; phytase; broiler chicken.

1. INTRODUCTION

Phytase (myo-inositol hexaphosphate phosphohydrolase) belongs to a specific group phosphatase that is capable of hydrolysing phytate to a series of lower phosphatase esters of myo-inositol and inorganic phosphate [1]. As more phosphorus is released from phytate leading to more breakdown of intact IP-6, the less able it is to bind or chelate minerals, starch or proteins either directly or via ionic bridges [2]. Reducing the phytate-bound compounds through the use of phytase may enhance the bioavailability and digestibility of phosphorus and divalent or trivalent cations but also indirectly increase energy metabolizability and nitrogen utilization [3]. Although environmental consideration is far below international standard in Nigeria, it is obvious that river, lakes and underground water contamination are environmental problems caused by the higher phosphorus elimination in the excretions. Such problems is even greater especially when bird manure is used as fertilizer since the amount of phosphorus added to soil exceeds plant requirements and excess phosphorus from faeces is easy to access ground water, rivers, lakes and oceans and can lead to mortality of aquatic animal by stimulating algae growth. According to [4] phosphorus and nitrogen are considered limiting elements for aquatic plant growth because their excess causes an increase of the eutrophication process and consequently reduction of the water quality. Unfortunately, monogastric animals do not possess phytase enzymes in quantities that would allow these animals to utilize phytate as a source of phosphorus. In the 1960's, a group of scientist working for international minerals and chemicals corporation recognised that the need to supplement the diets of monogastric animals with inorganic phosphorus could be decreased if phytate phosphorus could be made available to animals by treating the grains with phytase. For these mentioned reasons at present phytase is being used in poultry industry to enhance more nutrients and reduce pollution problems emanating from phosphorus in faeces. The study aimed to investigate the effect of phytase on haematological, serum metabolites and enzyme activities for evaluating animal's health status.

2. MATERIALS AND METHODS

The feeding trial carried out at the poultry unit of the Teaching and Research Farm of the Ekiti State University, Ado-Ekiti, Ekiti- state, Nigeria, Africa was conducted to assess phytase (Ronozyme-p) supplementation effect on blood, serum and enzyme activities by broiler chicken fed rations formulated using predominantly plant-based ingredients. The poultry house was thoroughly fumigated with one part of potassium permanganate pellets to three parts of formalin. The pen was allowed to stay for two weeks before the arrival of the experimental birds. A total number of two hundred and forty broiler birds of Anak 2000 commercial breed were used in the two separate experiments investigating the haematological parameter, serum metabolites and enzyme activities of broiler chickens.

2.1 Experimental Diets

The composition of five experimental diets were compounded on the floor at the premises of the Teaching and Research Farm, Ekiti State University, Ado-Ekiti, Nigeria, Africa The five diets were compounded to be iso-nitrogenous and iso-caloric. The ingredients were predominantly plant products. Diet 1 was the reference diet with no phytase supplementation. The protein sources in the reference diet were from both plant and animal origins. Diets 2 and 3 were duplicate diets with enzyme supplementation only in diet 3. Diets 4 and 5 were also duplicate diets with enzyme supplementation only in diet 5. Diets 2 and 3 had groundnut cake as the major plant protein ingredient while diets 4 and 5 had soybean meal as the major plant protein ingredient. All diets were supplemented with methionine and lysine.

2.2 Experimental Animals

A total of two hundred and forty day old unsexed chicks of commercial broiler strain (Anak 2000) were purchased from a reputable hatchery for this study. During brooding enough, provision was made for space, ventilation and protection against predators and extreme of weather. Prior to the arrival of the chicks, the brooder house and other equipment were thoroughly disinfected. The chicks were fed *ad-libitum.* There were five experimental diets per treatments with four replicates per treatment. Twelve birds were allotted per replicate, amounting to 48 birds per treatment. During the brooding exercise, the chicks were given all the recommended vaccination and medication.

2.3 Enzyme (Phytase Description and Inclusion in Diets)

A commercial phytase (Ronozyme-p) was purchased from Lagos, Nigeria, Africa. It is a granulated phytase produced from *Peniophora lycii* by submerge fermentation of a genetically modified *Aspergillus oryzae* micro-organism. The enzyme has a minimum activity of 250 FTU/G. The inclusion rate was 0.1g/kg for the experiment.

2.4 Collection of Blood Samples

Blood collection was carried out at the end 8th week of the experiment. This was done in the morning after the birds were starved overnight in order to attain a stable haematological evaluation. Two birds were randomly selected from each experimental diet and blood samples were collected from the birds through jugular veins at the termination of each experiment. About 5 mls of blood samples were collected from each bird in labelled bottles containing Ethylene Diaminetetra-acetic Acid (EDTA) to determine the haematological indices. The tubes were immediately capped and the contents mixed gently for about 1minute by repeated inversion.

Blood samples meant for serum metabolites and enzyme activities were collected in vacutainer tubes without anticoagulants and sent to the laboratory. The tubes were kept in a slanting wooden rack and the blood samples were allowed to clot overnight. The serum (supernatant) was separated clearly by decanting after the blood samples were spun in a centrifuge at 300 rpm for 10minutes. The serum samples were kept in sterile vacutainer tubes and kept deep frozen prior to analysis.

2.5 Analysis of Blood Samples

Packed cell volume (PCV) was determined by microhaematocrit method [5]. Haemoglobin (Hb) concentration was measured spectrophoto-metrically by cyanomethaemoglobin method [5,6] using Sp6-500 UV spectrophotometer (Pye UNICAM ENGLAND). The Red Blood Cell (RBC count were estimated using haemocytometer [5]. Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) were calculated from Hb, PVC and RBC [7]. Serum total protein was determined by Kjedahl method as described by [8] while albumin was determined using the BCG (Bromocresol green) method as described by [9]. Cholesterol was determined according to [10] while urea was determined as described by [11]. Aspartate Transaminase (AST) and Alanine Transaminase (ALT) activities were determined using spectrophotometric method as described by [12] and [13], respectively. Alkaline phosphatase, Creatinine, Triglyceride,High density lipoprotein and Low density lipoprotein were determined.

2.6 Statisical Analysis

All data collected in these studies were subjected to analysis of variance. Duncan's Multiple Range Test of one way ANOVA (SPSS 17.0 for windows, SPSS Inc., Chicago, IL, USA) was used to analyse the mean differences of the same parameters. Significant differences were considered where necessary at a level of (P=0.05).

3. RESULTS AND DISCUSSION

The results of the haematological indices revealed that PCV, RBC, HB of birds on reference diets were higher in values when compare to the test ingredients. The highest PCV (29.00±1.16)%, RBC (2.11±0.06) $\times 10^6 mm^3$, Hb (7.89±0.30) g/100ml were recorded for birds on reference diet while the least of these parameters (26.74±0.58%, 1.76±0.05 $\times 10^6 mm^3$, 7.20±0.35 g/100 ml), respectively was recorded for birds on diet 2 (groundnut based diet without phytase supplementation). However, PCV, RBC, HB values of all birds sampled among dietary

Table 1. Composition of experimental diets (g/100g) for broiler finisher birds (0-56days)

Ingredients	Diets				
	1	2	3	4	5
	Phytase inclusion				
	Reference diet	-	+	-	+
Maize	62.00	62.00	62.00	62.00	62.00
Groundnut cake	10.00	25.00	25.00	0.00	0.00
Soyabean	15.00	0.00	0.00	25.00	25.00
Wheat offal	6.70	8.70	8.70	8.70	8.70
Fishmeal (72% CP)	2.00	0.00	0.00	0.00	0.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Oyster shell	0.50	0.50	0.50	0.50	0.50
NaCl	0.50	0.50	0.50	0.50	0.50
Methionine	0.15	0.15	0.15	0.15	0.15
Lysine	0.15	0.15	0.15	0.15	0.15
Premix	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition					
Crude protein (g/100g)	19.88	18.93	18.93	18.68	18.68
Crude fibre (g/100g)	3.73	3.49	3.49	4.17	4.17
Metabolisable energy (Kcal/kg)	2935.87	2888.35	2888.35	2913.35	2913.35
Analysed composition					
Crude protein (g/100g)	21.16	20.48	20.47	20.76	20.74
Crude fibre (g/100g)	5.24	5.43	5.43	5.37	5.36

Negative sign (-) means no phytase inclusion; positive sign (+) means phytase inclusion at 250 FTU/g minimum activity inclusion rate of phytase in diets

treatments indicated that they were not significantly (P>0.05) affected by diets. The MCV and MCH of birds on experimental diets were not significantly (P>0.05) affected. The MCHC values of birds on diets 2, 3, 4 and 5 were similar (P>0.05) but significantly (P<0.05) higher than MCHC values of reference diets. The highest value of MCHC was recorded for birds on diet 3 at 30.04±1.83% while the least value was recorded for birds on reference diet at 21.49±0.77%. With the exception of eosinophil, the haematological variables (neutrophils, lymphocytes, basophils and monocyte) were similar and consistent, only the eosinophil decreased significantly (P<0.05) as compared to reference point.

Blood represents a means of assessing clinical and nutritional health status of animals in feeding trial and the haematological parameters (Table 2). Most commonly used haematological parameters are PCV, RBC, HB, MCHC, MCV and MCH [14,15,16]. The results of haematological variables in this study suggest that the test diets did not pose any severe effects on the health status of the experimental birds. Increased MCHC of birds on phytase supplemented diets could be as a result of agglutination of red blood cells (falsely lowering the measured RBC) or when there is opacifaction of the plasma (falsely increasing the measured haemoglobin). Causes of plasma opacification that can falsely increase the MCHC include hyperbilirubinemia, hypertriglyceridemia and free haemoglobin in the plasma (due to haemolysis).

With the exception of eosinophil, the haematological variables (neutrophils, lymphocytes, basophils and monocyte) were similar and consistent, only the eosinophil decreased significantly (P<0.05) when compared to reference diet. A low eosinophil level might be due to endogenous corticosteroid release. There may be increased circulating steroids because inositol may have effect on hormone regulation (thyroxine, prostaglandis). A low eosinophil level is common, some birds with low eosinophil levels live normal lives.

Table 3 showed that all the experimental diets had no significant influences on all biochemical parameters investigated in this experiment (P>0.05). However, dietary phytase supplementation had no effect on total protein concentration in serum. In this study, the

potential existence of protein-phytate complex in the gut of chick could affect the digestibility and absorption of protein [3] which modifies the concentrations of total protein in serum [17]. There was a slight increase in the values of triglyceride and high density lipoprotein, on the other hand, a decrease in the values was recorded in low density lipoprotein. These result of this study agreed with the report of [18] and [19]. They found that phytase supplementation had no adverse effect on biochemical constituents of broiler serum.

The effects of phytase supplementation on enzyme activities are presented in Table 4. Aspartate amino transferase (AST) activity increased (P<0.05) in the birds fed diets supplemented with phytase.The increased AST could be as a result of increase in cellular injury in most tissues or haemolytic disorders.

Table 2. Haematological indices of broiler finisher birds with or without exogenous phytase supplement (0-56day)

Parameters	Diets				
	1	2	3	4	5
		Phytase inclusion			
	Reference diet	-	+	-	+
PCV (%)	29.00±1.16	26.74±0.58	28.90±2.58	28.50±4.51	28.98±1.00
RBC(×$10^6$$mm^3$)	2.11±0.06	1.76±0.05	2.07±0.29	1.84±0.11	1.87±0.04
HBC(G/100ml)	7.89±0.30	7.20±0.35	7.25±0.27	6.88±0.72	7.32±0.05
MCV(µm^3)	155.46±3.22	156.12±6.00	156.65±19.34	156.30±10.29	155.13±427
MCHC (%)	21.49±0.77^c	26.20±1.83^{ab}	30.04±2.41^a	28.97±2.35^{ab}	29.74±2.30^a
MCH(pg)	34.39±0.74	34.00±2.25	34.43±0.59	34.45±2.64	34.52±2.16
Neutrophils (%)	38.00±2.83	37.50±1.95	38.50±6.61	37.48±4.93	38.14±1.00
Lymphocytes (%)	42.25±1.89	42.18±2.36	42.24±2.63	41.75±1.17	41.77±1.71
Eosinophils (%)	4.80±1.29^a	4.00±0.82^b	4.15±2.22^b	4.10±5.26^b	4.00±1.63^b
Basophils (%)	1.00±0.01	1.00±0.01	1.02±0.50	1.00±0.58	0.97±0.01
Monocytes (%)	7.390±1.00	7.50±1.00	7.00±1.00	7.49±4.44	7.50±1.00

Means with different superscript on the same row differ significantly (p<0.05)
Negative sign (-) means no phytase inclusion; positive sign (+) phytase inclusion at 250 FTU/g minimum activities inclusion rate of phytase in diet. PCV = Packed cell volume; RBC =Red blood cell; HBC; Haemoglobin Concentration, MCV = Mean corpuscular volume; MCHC = Mean corpuscular haemoglobin concentration; MCH = Mean corpuscular haemoglobin.

Table 3. Serum metabolites of broiler finisher birds fed diets with or without exogenous phytase supplement (0-56day)

Parameters	Diets				
	1	2	3	4	5
			Phytase inclusion		
	Reference diet	-	+	-	+
Total serum protein (g/100ml)	25.20±1.40	24.21±0.29	24.14±0.12	24.04±0.33	24.16±0.19
Albumin(g/100ml)	11.12±0.48	10.80±1.22	11.23±1.50	11.90±0.26	11.20±1.22
Globulin(g/100ml)	14.58±0.99	14.41±1.23	14.92±1.38	14.86±0.63	14.76±1.29
Albumin/Globulin	0.82±0.08	0.74±0.12	0.76±0.16	0.77±0.04	0.77±0.14
Urea (mmol/L)	0.88±0.14	0.84±0.09	0.82±0.06	0.81±0.11	0.85±0.07
Creatinine (mg/dl)	1.65±0.76	1.64±1.14	1.64±0.97	1.63±1.12	1.64±1.90
Total cholesterol (mg/dl)	78.80±0.34	77.65±0.06	76.87±0.13	76.56±0.06	76.43±0.05
Triglyceride	0.28±0.05	0.25±0.56	0.28±0.13	0.26±0.05	0.29±0.05
High density lipo protein	85.47±0.66	84.98±0.10	84.10±0.12	84.15±0.13	83.89±0.43
Low density lipo protein	19.50±0.03	19.25±0.11	18.96±0.13	18.75.±0.20	18.78±0.09

Means with different superscript on the same row differ significantly (<0.05)
Negative sign (-) means no phytase inclusion;
positive sign (+) phytase inclusion at 250 FTU/g minimum activities inclusion rate of phytase in diet.

Table 4. Enzyme activities of broiler finisher birds fed diets with or without exogenous phytase supplement (0-56 day)

Parameters	Diets				
	1	2	3	4	5
			Phytase inclusion		
	Reference diet	-	+	-	+
Aspartate amino transferase (mmol/L)	99.75±1.50[b]	99.00±0.01[b]	103.50±1.01[a]	100.00±0.01[b]	105.00±0.01[a]
Alkaline phosphatase(mmol/L)	125.44.±0.62[a]	120.19±0.79[b]	116.42±3.73[c]	123.84±1.6[ab]	118.80±1.51[c]
Alanine amino transferase(mmol/L)	105.45±0.04	104.74±0.12	102.46±2.02	103.72±0.03	102.23±0.03
Ɣ-glutamyl transferase(mmol/L)	53.45±0.04	52.86±0.12	53.18±2.02	51.98±0.03	52.78±0.07

Means with different superscript on the same row differ significantly (p<0.05) Negative sign (-) means no phytase inclusion; positive sign (+) phytase inclusion at 250 FTU/g minimum activities inclusion rate of phytase in diet.

The alkaline phosphatase (ALP) activities of birds on diets supplemented with phytase was significantly (P<0.05) reduced when compared to ALP of birds on non-phytase supplemented diets. The decrease in ALP activity associated with diets supplemented with phytase might be as a result of the down regulation of phytase which resulted from the increase in availability of phosphorus released from the diet [20]. [21] reported that decreased values of alkaline phosphatase is not clinically significant. Alanine amino transferase (ALT) activities decreased in birds fed diets supplemented with phytase. ALT activities has be reported to be low in all tissues of chicken [22]. [23] reported that ALT activities often increase due to damage in many tissues. Gamma-glutamyl transferase (GGT) of birds on experimental diets were not affected by the dietary phytase supplementation.

4. CONCLUSION

A low eosinophil level was recorded in haematological parameters this could be due to inositol which may have effect on hormone regulation. Phytase supplement had no effect on serum metabolites though there were changes in aspartate amino transferase and alkaline phosphatase. From this study it was concluded that parameters investigated did not indicate any changes that would suggest that phytase supplementation in broiler's diets affected the health of broiler chickens. Furthermore, the results obtained in this study revealed that phytase supplementation had no side effect on haematological parameters, biochemical constituents at serum level and enzyme activities rather, it modifies some serum enzyme activities.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Sebastian S, Touchburn SP, Chavez ER. Implications of phytic acid and supplemented microbial phytase in poultry nutrition: A Review. World Poultry Journal. 1998;54:27-47.
2. Selle PH, Ravindran V. Microbial phytase in poultry nutrition. Animal Feed Science and Technology. 2007;135:1-41.
3. Selle PH, Ravindran V, Cadwell RA, Bryden WL. Phytate and phytase. Consequences for protein utilization. Nutr. Res. Rev. 2000;13:255-278.
4. Syers JK, Harris RF, Armstrong DE. Phosphate chemistry in lake sediments. Journal Environ. Quality. 1973;2:2-14.
5. Schalm JW, Jain NC, Carol EJ. Veterinary haematology. 3rd Edn. Lea and Febriger. Philadelphia USA. 1975;15-81.
6. Swenson MT. Effects of vitamin B_{12} and livermeal on the haematology of chicks fed on low protein in ration. Anim. Vet. Res. 1951;12:147-151.
7. Jain NC. Schalm's veterinary haematology. 4th Edn. Lea and Febrigen, Philadephia, USA, pp.34-50.Kaplan, A. & Szabo, I.I. (1979). Clinical chemistry. Interpretation and technique. 1st Edn. Henry Kumpton Publishers. London. 1986;109-110.
8. Kohn RA, Allen MS. Enrichment of proteolysis activity relative to nitrogen in

preparations from the rumen for in-vitro studies. Anim. Feed Sci. Technol. 1995;52:1-4.
9. Peters T, Biamonte GT, Doumas BT. Protein (total protein) in serum. In: Selected methods applications to livestock management and of clinical chemistry. Faulkner GWR, Mcites S. (Eds.). American Association of clinical chemistry. 1982;100-115.
10. Roschlan P, Bernet E, Guber W. Enzymatische bestimmung des gesant cholesterium in serum. Journal Clin. Biochem. 1974;12:403-407.
11. Kaplan A, Szabo II. Clinical chemistry: Interpretation and technique. 1st Edn. Henry Kumpton Publisher, London. 1979;109-110.
12. Rej R, Holder M. Aspartate amino transferase. In: Methods of enzymatic analysis. 3rd Edn. Bergmeyer Hu, Bergmeyer, J., Grassl, M. (Eds.) Weinheiin Velagchemie. 1983;416-433.
13. Holder M, Rej R. Alanine transaminase. In methods of enzymatics analysis. 1983; 3rd Edn.
14. Aletor VA, Adeogun OA. Nutrients and anti-nutrient components of some tropical leafy vegetables. Food Chemistry. 1995; 54(4):375-379.
15. Olorede BR, Longe OG. Effects of replacing palm kernel cake with shea butter cake on quality characteristics, haematology and serum chemistry of laying hens. Nigeria Journal of Animal Production. 2000;27:19-23.
16. Adeyemi OA, Fasina OE, Balogun MO. Utilization of full fat jatropha seeds in broiler diet: Effect on haematological parameters and blood chemistry. Proceedings of the 25th conference of Nigeria Society for Animal Production held at Micheal Okpara University of Agriculture, Umudike, Nigeria. 2000;108-109.
17. Underwood, E.J and Suttle, N.F. In: The mineral nutrition of livestock 3rd Edn. 1990;105-106. CABI, Wallingford, UK.
18. Shnakmak S. Improvement of productive performance in poultry. M.Sc Thesis Mansour University; 2003.
19. Al-Harti MA. Impact of supplemental feed enzymes condiments mixture of their combination on broiler performance, nutrient digestibility and plasma constituents. International Journal of Poultry Science. 2006;5(8):764-771.
20. Huff WE, Moore PA, Waldroup PW, Waldroup AL, Balog JM, Huff GR, Rath NC, Daniel TC, Raboys V. Effects of dietary phytase and high non-phytate phosphorus corn on broiler chicken performance. Poult. Sci. 1998;77:1899-1904.
21. Peter ML, Susan CEF. Interpretation of laboratory results. Aust. Vet. Pract. Timer. 1991;21(4):188-193.
22. Bogin E, Israeli B. Enzymes profile of heart and skeletal muscle, liver and lung of rooster and geese. Zbl. Vet. Med. A. 1976;23:152-157.
23. Zantop DW. Biochemistries. In: Avian medicine: Principles and Applications. 1997;115-129. B.W. Ritchie, G.J. Harrison and L.R Harrison, (ed.) Wingers publishing Inc., Lakeworth, FL.

Peer-review history:
The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/21198