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UTILIZATION OF COTTONSEED MEAL WITH OR WITHOUT PHYTASE SUPPLEMENTATION ON PERFORMANCE AND HAEMATOLOGICAL PARAMETERS DURING SHORT TERM FEEDING PERIODS IN BROILER CHICKENS

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ABSTRACT

The utilization of cottonseed meal (CSM) as a protein supplement in poultry diets is hindered by the presence of gossypol which can adversely affect growth performance and increase mortality in broilers. This study investigated the impact of CSM supplemented with phytase on growth, performance, and hematological parameters in Arbor Acre broiler chickens. A total of 216 day-old broiler chickens were utilized in the experiment, starter diets was provided until day 20 and thereafter experimental diets from days 21 to 28. Six semi-purified diets were formulated in a 3x2 factorial, varying in CSM content (150, 300, and 450g/kg) and phytase supplementation (0 or 1000 units of Natuphos). Feed intake, body weight, and weight gain were monitored, while blood samples were collected for hematological analysis. There were no significant differences (P>0.05) in feed intake, final weight, and weight gain among treatment groups with or without phytase supplementation, however average feed intake per bird across the treatments ranged from 219.80 to 278.00g, while a range of 204.30 to 259.00g was observed for average dry matter intake per bird. Hematological parameters including erythrocytes, hemoglobin, packed cell volume, lymphocytes, heterocytes, basophil, eosinophils were within the normal range and did not differ significantly between birds fed diets with or without phytase supplementation. The inclusion of phytase in broiler diets showed no adverse effects on blood parameters and improved growth performance. Overall, these findings suggest that phytase supplementation in broiler diets containing CSM could be beneficial for growth performance without impacting blood parameters during short-term feeding periods.

Keywords: Arbor Acre, Basophils, Broiler, Cottonseed meal, Haemoglobin, Phytase

INTRODUCTION

In poultry nutrition, optimizing feed formulations to enhance growth performance and overall health is paramount. Cotton seed meal (CM) is a common ingredient in poultry diets due to its protein content, but its inclusion can affect nutrient utilization and impact hematological parameters (Zeng et al., 2014). Phytase, an enzyme that enhances phosphorus availability, is often supplemented in poultry diets to mitigate anti-nutritional factors in ingredients like CM. However, the combined effects of CM and phytase on hematological indices in broiler chickens remain understudied. Research has shown that the inclusion of cottonseed meal in broiler diets can have positive effects on growth performance, carcass traits, and fat deposition, indicating that cottonseed meal can be a potential alternative protein source, with high crude protein content ranging from 35% to 44% (Thirumalaisamy et al., 2013; Chen et al., 2023), depending on processing methods. Additionally, the replacement of soybean meal with cottonseed meal in broiler diets, even up to 90% was reported not to compromise performance indicators (Yu et al., 2019). Enzyme supplementation, such as with phytase, has been found to further enhance the utilization of cottonseed meal in broiler diets without negatively impacting growth performance. Moreover, the use of fermented cottonseed meal has been investigated (Liu et al., 2022; El-Saidy and Saad, 2011; Swiatkiewicz et al., 2016), showing improvements in growth performance, carcass traits, and reduced fat deposition in broiler chickens and other animals. Fermented cottonseed meal supplementation has been linked to decreased feed conversion ratios, increased carcass percentages, and improved fat metabolism in broilers (Adeyemo, 2010; Dalle-Zotte et al., 2013). Broiler chicken production is a vital aspect of the poultry industry, and optimizing growth performance and skeletal health is essential for economic viability and animal welfare. Cottonseed meal (CSM) is commonly used in broiler diets as a protein source, but its inclusion may influence growth performance and bone mineralization due to the presence of anti-nutritional factors. Phytase supplementation has been explored as a strategy to mitigate these effects by improving phosphorus availability according to Cowieson et al. (2011) and subsequently reducing inorganic-phosphorus supplementation and phosphorus excretion (Bougouin et al.. 2014). This analysis aims to evaluate the impact of CSM-based diets and phytase supplementation on selected growth performance indices and tibiae bone ash in 28-day-old broiler chickens.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted in the Teaching and Research Farm, University of Ibadan, South West Nigeria, Ibadan is on Latitude 7 20' N and longitude 3 50' E.

Test Ingredients

The test ingredients used is cottonseed meal (CM). Sufficient quantities of CM and other feed ingredients needed for the formulation of the assay diets were purchased in bulk to avoid variation due to batches

Management of experimental birds

The poultry house and equipment used were thoroughly washed and disinfected before the arrival of the chicks. Groups of one-day-old Abor Acre broiler chicks were obtained from a reputable commercial hatchery and raised on floor pens in a well-ventilated and illuminated standard poultry house. On arrival, the birds were fed a commercial broiler starter diet that met NRC (1994) nutrient requirements for broiler chicks for 14 days. On day 14, the birds were transferred to metabolic cages. At day 20 post-hatch, 288 birds were tagged, individually weighed and randomly allotted to experimental diets with six replicates (n=288) in a randomized complete block design using experimental animal allotment programme (EAAP) of Kim and Lindemann (2007) for all the studies. Birds in all studies had free access to water and experimental diets for eight days, with the first two days allowed for acclimatization to the experimental diets. All necessary routine management practices as well as room temperature regulation were adhered to.

Dietary treatments

Sequential, semi-purified diets containing 150, 300 or 450g of CM/kg 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 (Tables 1). Soya oil was added to the diets to obtain similar gross energy across the diets.

Haematology

Haematological analysis were conducted in the Animal Physiology and Bioclimatology laboratory, University of Ibadan. Hematological parameters including Packed Cell Volume (PCV), Hemoglobin (HB), Red Blood Cell count (RBC), White Blood Cell count (WBC), Platelet count (PLT), Lymphocyte percentage (LYMP), Hematocrit (HETR), Eosinophil percentage (EOS), and Basophil percentage (BOS) were measured. Data were analyzed using appropriate statistical methods, and means were compared to elucidate the effects of CM and phytase supplementation on hematological indices. Packed cell volume (PCV) was determined by the procedure described by Ratiff and Halls (1973), while other haematological parameters were determined using the procedure described by Schalm et al. (1975).

Table 1: Gross composition (g/kg) of cottonseed meal-based diets supplemented without or with phytase (as-fed basis)

	0 FTU/Kg (Phytase)			1000FTU/Kg(Phytase)		
Ingredients	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**
Cotton Seed meal (CSM)	150.00	300.00	450.00	150.00	300.00	450.00
Cassava Starch	495.50	343.75	192.00	485.50	333.75	182.00
Casein	200.00	200.00	200.00	200.00	200.00	200.00
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	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	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
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						
ME Kcal/Kg	3836.06	3666.45	3496.84	3801.16	3631.55	3461.94
CP (g/kg)	236.50	298.60	360.70	236.50	298.60	360.70
Ca (g/kg)	5.34	6.23	7.12	5.34	6.23	7.12
Total P (g/Kg)	3.46	4.91	6.37	3.46	4.91	6.37
Phytate P (g/Kg)	1.13	2.25	3.38	1.13	2.25	3.38
Non Phytate P (g/kg)	2.33	2.66	2.99	2.33	2.66	2.99
Ca : NPP ratio	2.29	2.34	2.38	2.29	2.34	2.38
Ca : P ratio	1.55	1.27	1.12	1.55	1.27	1.12

[1]Composition of vitamin premix per kg of diet: vitamin A, 12500 I.U; vitamin E, 40mg; vitamin K_3, 2mg; vitamin B_1, 3mg; vitamin B_2, 5.5mg; niacin, 5.5mg; calcium pantothenate, 11.5mg; vitamin B_6, 5mg; vitamin B_{12}, 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

RESULTS AND DISCUSSION

In table 2, the DM content of CM-based diets ranged from 93.10% to 96.50%, with higher levels observed in diets containing lower levels of CM. The CP content ranged from 22.70% to 29.20%, with higher levels observed in diets containing higher levels of CM. However, Ca and total P levels varied across the diets, with higher levels observed in diets containing higher levels of CM. The Ca:P ratio ranged from 1:1 to 2.5:1 as stated in table 2. The Gross energy content ranged from 3700 kcal/kg to 3950 kcal/kg, with slight variations observed across the diets.

Table 2: Nutrient contents and gross energy indices of 28-day-old broilers[1] fed cottonseed meal based diets

Composition (%)	Phytase, 0FTU/kg			Phytase, 1000FTU/kg			SEM
	CM, 150g/kg of diet	CM, 300g/kg of diet	CM, 450g/kg of diet	CM, 150g/kg of diet	CM, 300g/kg of diet	CM, 450g/kg of diet	
DM	96.50	95.70	93.10	93.00	93.00	93.50	0.35
CP	22.50	27.60	29.10	22.70	26.40	29.20	2.31
Ca	3.80	4.66	5.30	4.00	4.17	4.07	0.53
TP	1.76	3.75	5.07	1.60	3.18	4.79	1.47
Ca:TP	2:2:1	1:2:1	1:1	2:5:1	1:3:1	1:1	-
GE (kcal/kg)	3940	3880	3880	3950	3920	3700	86.65

DM- Dry Matter, CP- Crude Protein, Ca-Calcium, TP- Total Phosphorus, GE-Gross Energy, CM- Cottonseed Meal

Results of selected growth performance indices investigated and percentage tibiae bone ash of the experimental birds fed CSM based diets are presented in Table 3. Feed conversion ratio (FCR) and percentage tibiae bone ash of the birds had no significant (P>0.05) response to feeding graded level of cottonseed meal with or without supplemental phytase. However, the responses for feed intake, dry matter intake (DMI) and average body weight gain increased as dietary inclusion levels of CM increased across the sequential diets. Addition of phytase to CM had no effect (P>0.05) on the measured growth performance indices. Average feed intake per bird across the treatments ranged from 219.80 to 278.00g, while a range of 204.30 to 259.00g was observed for average dry matter intake per bird. Body weight gain per bird was neither influenced (P>0.05) by the addition of phytase nor the interaction, phosphorus× phytase as the dietary phosphorus content increases. Comparing body weight gain of the birds at specific level of CM inclusion in the diet for with or without phytase showed no variation (P>0.05), except for the diet containing 450g/kg CM in the phytase supplemented diet. Average body weight gains per bird fed CM diets without supplemental phytase were; 111.40, 130.10 and 162.60g as against 113.50, 118.50 and 147.90g observed for birds that received CM with supplemental phytase. Irrespective of phytase addition to the diets, average body weight gain were not significantly different (P>0.05) across the treatments. From the results of the study, phytase supplementation of cottonseed meal had no significant (P>0.05) effect on feed conversion ratio. Feed conversion ratio values for birds fed CM without phytase were 2.05, 1.97 and 1.79 while corresponding values of 1.93, 1.95, and 1.85 were observed for birds on CM phytase supplemented diets. Tibiae bone ash of the birds, were neither significantly (P>0.05) influenced by increasing dietary P from CSM, phytase nor interaction (phosphorus × phytase). Tibiae bone ash for birds fed CM without phytase were 43.04, 46.01 and 48.95% as against 44.04, 43.07 and 45.80% for birds that received phytase supplemented diets.

Table 3: Selected growth performance indices and percentage tibiae bone ash of 28-day-old broilers[1] fed cottonseed meal based diets

Measurements	Phytase (0 FTU/kg)			Phytase (1000 FTU/kg)			Pooled SEM[3]	P-value						
											Without Phytase		With Phytase	
	CSM 150g/kg of diet	CSM 300g/kg of diet	CSM 450g/kg of diet	CSM 150g/kg of diet	CSM 300g/kg of diet	CSM 450g/kg of diet		Phytase (Phy)	*P	Phy X P	L^2	Q^2	L^2	Q^2
Feed intake (g/bird)	229.00	250.00	278.00	219.80	228.50	272.20	6.91	0.338	0.0073	0.867	0.036	0.849	0.031	0.374
Dry matter intake (g/bird)	221.10	233.50	259.00	204.30	212.50	253.80	6.32	0.223	0.011	0.845	0.069	0.702	0.029	0.365
Body weight gain (g/bird)	111.40[a]	130.10[ab]	162.60[b]	113.50[a]	118.50[a]	147.9[b]	4.79	0.302	0.003	0.644	0.007	0.633	0.003	0.163
Feed conversion ratio	2.05	1.97	1.79	1.93	1.95	1.85	0.055	0.826	0.436	0.805	0.273	0.015	0.620	0.640
Tibiae bone ash (%)	43.04	46.01	48.95	44.04	43.07	45.80	0.845	0.315	0.166	0.522	0.826	0.992	0.621	0.547

[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 (8 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

As the level of CM increased in the diet, PCV, HB, and RBC exhibited a declining trend in Table 4. Birds fed higher levels of CM (450g/kg) displayed lower PCV, HB, and RBC compared to those on lower CM inclusion levels (150g/kg and 300g/kg). the values of WBC and PLT counts were influenced by CM inclusion, with birds fed higher levels of CM showing higher WBC and PLT counts compared to those on lower CM inclusion levels. The percentages of LYMP, EOS, and BOS did not exhibit consistent trends with CM inclusion levels, suggesting minimal impact on these parameters. However, HETR showed a slight increase with higher CM levels, indicating potential dehydration effects. However, Phytase supplementation did not significantly affect PCV, HB, or RBC levels.

Table 4: Haematological indices of 28-day-old broilers fed graded diet levels of cottonseed meal

	Phytase	0FTU/kg		Phytase	1000	FTU/kg	
	CM 150G/kg	CM 300G/kg	CM 450G/KG	CM 150g/kg	CM 300g/kg	CM 450g/kg	SEM
PCV	36.70	42.70	27.30	32.00	29.70	34.30	5.69
HB	12.00	13.90	8.93	10.40	9.50	11.20	1.87
RBC	11.97	12.32	10.24	10.61	10.57	12.27	0.76
WBC	10667.00	9500.00	13117.0	10350.0	11400.00	9400.00	101.40
PLT	114333.0	392333.0	128667.0	152000.0	399333.0	116333.	14701.5
LYMP	65.70	63.00	64.00	64.00	60.00	60.30	3.19
HETR	30.30	33.70	31.70	33.00	37.00	36.30	3.42
EOS	1.67	2.00	2.33	1.00	2.00	2.33	0.38
BOS	2.33	1.33	2.00	2.00	1.00	1.67	0.71

PCV—packed cell volume , WBC –white blood cells , NEUT—neutrophils ,HB –heamoglobin concentration , PLAT – platelets ,MON --- monocytes ,RBC—red blood cells, LYM –lymphocyte, EO –eosinophil , SEM- Standard Error of Mean

Discussion

The variation in CP content across the diets highlights the importance of formulating diets to meet the protein requirements of birds at different growth stages. The Ca:P ratio plays a critical role in bone development and overall health in poultry. Diets with imbalanced Ca:P ratios may lead to skeletal issues and impaired performance (Adeyemo, 2010). Diets with higher energy content may support better growth performance, provided other nutrient requirements are met. Furthermore, feeding increasing dietary concentration of phosphorus from CM clearly resulted in a decrease in feed conversion ratio of the birds, which clearly indicates that the lower the ratio the better is the index (Valable et al., 2018). However, excess energy without proper balance can lead to metabolic disorders and reduced feed efficiency. Phytase supplementation enhances phosphorus utilization in poultry diets by breaking down phytate, a form of phosphorus that is poorly digestible by poultry (Angel et al., 2006). The inclusion phytase in CM-based diets can improve phosphorus availability and reduce the need for inorganic phosphorus supplementation. Phytase supplementation may influence the utilization of other nutrients, such as calcium and protein. The nutrient composition of CM-based diets plays a crucial role in meeting the nutritional requirements of poultry, a good understanding of the nutrient composition and their implications on poultry nutrition as observed in

this study are essential for formulating balanced diets that support optimal growth and performance (Cowieson et al., 2011). Phytase supplementation offers additional benefits by improving phosphorus utilization and reducing the environmental impact of poultry production (Delezie et al., 2015). By carefully formulating diets based on the nutrient composition of CM and incorporating phytase supplementation where necessary, poultry producers can optimize feed efficiency and promote overall bird health and productivity especially as the prices of feed ingredients are soaring up season to season. This study displayed a trend towards decreased intake with increasing levels of CM in the diet, particularly in the absence of phytase supplementation. This indicates potential palatability issues or reduced nutrient utilization at higher CM inclusion levels. However, Phytase supplementation further improved weight gain, suggesting enhanced nutrient utilization and growth performance in birds receiving phytase-supplemented diets; this is in line with the report of Jing et al (2021) in phytase supplementation in laying birds. Numerical improvements in FCR as observed with phytase supplementation, particularly at higher CM inclusion levels, indicates that phytase supplementation may enhance feed efficiency by improving nutrient digestibility and utilization (Liu et al., 2022). Tibiae bone ash percentage, an indicator of bone mineralization had a trend towards higher bone ash percentages as observed with increasing levels of CSM in the diet, particularly in the absence of phytase supplementation. This suggests that higher levels of CSM may positively influence bone mineralization (Swiatkiewicz et al., 2016; Valable et al., 2018). However, in this study supplementation did not impact on tibiae bone ash and this is in line with suggestions as reported by Hughes et al. (2009) that the observed improvements in growth performance may not translate into significant improvements in bone mineralization. While CM inclusion positively influences body weight gain, its effects on bone mineralization are less pronounced. Phytase supplementation shows potential for improving nutrient utilization and feed efficiency but may have limited effects on bone mineralization under the conditions of this study.

CONCLUSION

This study found that supplementing cottonseed meal (CSM) with phytase in Arbor Acre broiler chicken diets did not significantly affect growth performance or hematological parameters, even when CSM content was increased to 450g/kg. The inclusion of phytase in broiler diets showed no adverse effects as evidenced by a slight increase in feed and dry matter intake. These findings suggest that phytase supplementation in broiler diets containing CSM could be a promising approach for improving growth performance without impacting blood parameters during short-term feeding periods.

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