GROWTH PERFORMANCE AND HEMATOLOGICAL PARAMETERS OF BROILER CHICKENS FED DIFFERENTLY PROCESSED SEED OF AFRICAN YAM BEAN (SPHENOSTYLIS STENOCARPA)

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Abstract

Three experiments were conducted to evaluate the growth performance and Hematological Parameters of Africa yam bean (Sphenostylis stenocarpa) in broiler chicken diets. In the experiment, different levels of (20, 40 and 60 %) of boiled AYB seeds were incorporated in broiler chicken diets as replacement for soyabean. The inclusion of AYB Significantly (P<0.05) influenced the performance characteristics indices of broilers. At the starter phase significant (p<0.05) differences existed in weight gain, feed consumption and final weight of broiler chickens. Average daily feed consumption values of 44.5, 44.9, 42.6 and 41.7 were significantly (p<0.05) affected at starter phase. Whereas at finisher phase, all others except daily feed intake, PER and mortality were significantly (p<0.05) affected by treatment effect. The highest weight of spleen was in 40% inclusion level. All the hematological indices and the serum chemistry of broiler indicated no significant (p>0.05) difference. Other parameters investigated include total protein, albumin, globulin, creatinine and urea. The serum cholesterol was however decreased significantly (p<0.05) as the level of AYB in the diets increased.

Introduction

The Nigerian poultry industry is estimated at ₦80 billion ($600 million) and is comprised of approximately 165 million birds, which produced 650,000 MT of eggs and 290,000 MT of poultry meat in 2013. From a market size perspective, Nigeria's egg production is the largest in Africa (South Africa is the next largest at 540,000 MT of eggs) and it has the 2nd largest chicken population after South Africa's 200 million birds. Livestock sector is vital to the socio –economic development of Nigeria. According to the Food and Agricultural Organization of the United Nations (FAO), "growing populations, economies and incomes are fuelling an ongoing trend towards higher consumption of animal protein in developing countries." FAO forecasts that Nigerians are expected to consume two thirds more animal protein, with meat consumption rising nearly 73%. This growth in protein consumption will drive demand for maize and soybeans, which form the core component of animal feed, Sahel (2015). Hence the search for cheap sources of protein-rich foods, increasing attention has been focused on home-grown under-exploited crops whose seeds contain relatively high amounts of protein that can be used to improve the diets of vast majority of populace.

Chicken importation (with the exception of day-old-chicks) was banned by Nigeria in 2003, which spurred growth in domestic poultry production. Statistics from Eurostat, however, high-light that between 2009 and 2011 over 3 million MT worth of poultry products were imported into the Republic of Benin, with the preponderance of these products ending up in the Nigerian market. Overall assumptions estimated poultry meat consumption in Nigeria is approximately 1.41, Ghana, 7.59, South Africa 32.98, Brasil, 31.34 and USA, 45.49 (kg/capita) {OECD,data 2014}. According to Ibe (2004) many Nigerians consume less than 10g of animal protein daily as against the minimum of 28g/caput/day considered consistent with a balance diet. More so, FAO (1986) estimated about 89.5g of protein essential for normal functioning of the body on a daily basis of which 34g be obtained from animal protein. Literature has proved that the consumption of meat indicates a position of social and economic position. It is noteworthy that meat consumption is often an indication of economic status of a country or individual (Ososanya, 2004). In classification of countries as either developed or developing, two major criteria are often used: per capital income and per caput animal protein consumption. Hence, there is need to intensify effort in livestock production.

The food deficit situation is indeed more serious with protein deficiency when compared to the availability of calories and the micro-elements. The problem of protein mal-nutrition persists in Africa and animal protein is very important in human nutrition since it contains the essential amino acids which are more balanced and readily available to meet human dietary requirements than protein of plant origin (Oyenuga, 1974).

The most costly portion of livestock foods are the protein and energy sources. In order to reduce the cost of poultry production without any adverse effect on the performance of the birds while maintaining an efficient feed utilization, there is need to source for alternative sources of protein and energy to replace the more costly ones.

The grain legumes contain significant amount of phosphorus and substantial amount of essential minerals and vitamins specifically the B-complex vitamins needed for proper growth and development of the body.

Therefore, the successful use of this legume as food for animals will reduce the over dependence on the well-known grain legumes such as soya bean and groundnut cake as plant protein sources and may eventually result to a decrease in the cost of protein sources and livestock feeds.

Materials and Methods

Experimental site:

The feeding trial will be conducted at the Teaching and Research Farm of the School of Vocational and Technical Education, College of Education Igueben, Edo state.

Sources of ingredients

The African yam bean samples (black) will be purchased from local farmers from Esan West, Igueben and Etsako Central local Government Areas in Edo State, Nigeria.

Processing of African yam bean seeds:

AYB seeds will be pre-soaked in batches of 10kg each for 12 hours to facilitate bean hydration before boiling. The bean samples will be boiled at 100-120^{0}C for 90minutes, using aluminum pot. The boiling water will not be changed. They will be considered boiled when they became soft when pressed between fingers. The residual boiling water will be drained off and the sample oven-dried over night at 75^{0}C and later sundried.

Experimental diets

In the experiment, four (4) starter and finisher diets will be formulated using boiled African yam bean meal (AYBM) since, the results from the rat experiment indicated that biological value (BV) will be highest in boiled AYBM. Diet 1 contained the conventional soya bean meal (SBM) based diet included in the study as a reference (0% AYBM). In diets 2, 3 and 4, boiled AYBM will be included at different levels of 20, 40 and 60% respectively. Other ingredients, included in the diets will be maize, fish meal, wheat bran, blood meal, palm oil, bone meal, oyster shell, vitamin premix, salt, lysine and methionine. The composition of experimental diets will be determined on dry matter basis. Both the starter and finisher diets will be constituted to be isonitrogenous and isocaloric with crude protein contents of 23% and 21%respctively (Tables 1 and 2).

Experimental design, chicken and their management

A total of 100 day old broiler chicks will be purchased from CHI poultry hatchery in Oyo State, Nigeria. All experimental chicks (96 day old chicks) will be electrically brooded and will be occasionally supplemented with kerosene lantern and stoves. Feeds will be supplied in conical metal feeders (modified) with less wastage; improved productive performance (Oluwasami *et. al.* 2006) and higher economic returns. All the chicks will be raised on four experimental starter and finisher diets: broiler-starter diets containing 23% protein for the first four (4) weeks and finisher diet containing 21% protein from fifth (5) week to eight (8) week. Prior to the arrival of the chicks, the experimental pen and other equipment will be properly disinfected. The chicks will be weighed and randomly allotted to the dietary treatments (1, 2, 3 and 4) in a completely randomized design (CRD) with four replicates each per treatment. At day−old, all the chicks will be randomly assigned at the rate of 6 chicks to each replicate (24 chicks per diet) in four (4) replications. This will be done in such a way that the average weights of chicks per diet will be equal. Feeds and water will be provided *ad libitum* until they are eight (8) weeks old. During the feeding trial, the chicks will be housed in deep litter floor with adequate provision for heat during the brooding period, good spacing, ventilation and protection against predators and other environmental conditions. They will be vaccinated against New castle and infectious bursal diseases and other routine management of broiler chicks will be also carried out.

Performance characteristics of the chickens in terms of weight gain, feed consumption, feed conversion efficiency, protein efficiency ratio (PER) and mortality will be investigated.

Table 1: Composition (g/100g) of broiler starter diets (0-4weeks).

DIETS	1	2	3	4
	(%) levels of AYB inclusion as replacement for SBM protein			
Ingredients	0	20	40	60
Maize	57.0	43.0	25.5	10.5
Soyabean meal (SBM)	29.5	19.0	12.0	5.0
African Yam bean meal (AYBM)	-	20.0	40.0	60.0
Wheat bran	6.3	6.3	6.3	6.3
Fish meal	3.5	3.5	3.5	3.5

Blood meal	-	2.5	3.0	3.5
Palm oil	-	2.0	6.0	7.5
Bone meal	2.5	2.5	2.5	2.5
Premix (min&vit)	0.3	0.3	0.3	0.3
Salt	0.3	0.3	0.3	0.3
Oyster shell	0.5	0.5	0.5	0.5
Lysine	-	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1
Total	100	100	100	100
Calculated CP	23.8	23.9	23.7	23.9
Calculated Metabolizable Energy (M.E) (kcal/kg)	2957.7	2951.6	2845.8	2895.6

Table 2: Composition (g/100g) of broiler finisher diets (5-8weeks)

DIETS	1	2	3	4
	(%) levels of AYB inclusion as replacement for SBM protein			
Ingredients	1	20	40	60
Maize	57.0	43.0	25.5	9.4
Soyabean meal (SBM)	23.5	12.9	5.9	3.5
African Yam bean meal (AYBM)	-	20.0	40.0	60.0
Wheat bran	12.4	12.4	12.4	12.4
Fish meal	3.5	3.5	3.5	3.5
Blood meal	-	2.5	3.0	3.4
Palm oil	-	2.0	6.0	7.5
Bone meal	2.5	2.5	2.5	2.5
Premix (min&vit)	0.3	0.3	0.3	0.3
Salt	0.3	0.3	0.3	0.3
Oyster shell	0.5	0.5	0.5	0.5
Lysine	-	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1
Total	100	100	100	100
Calculated CP	20.8	20.9	20.7	20.9
Calculated Metabolizable Energy (M.E) (kcal/kg)	2957.6	2951.6	2845.8	2895.6

Growth performance data collection

During the feeding trial, weekly feed consumed and average weight changes will be recorded, while weight gain, feed conversion ratio and protein efficiency ratio will be estimated to determine growth responses.

$$\text{Feed efficiency (g)} = \frac{\text{Feed consumed}}{\text{Weight gain}}$$

$$\text{Protein consumed (g)} = \frac{\%\ \text{protein in diet}}{100} \times \frac{\text{quantity consumed}}{1}$$

$$\text{Digestibility} = \frac{N_2\text{absorbed}}{N_2\text{intake}} \times \frac{100}{1}$$

Net protein Utilization (NPU) = Digestibility × biological value.

$$\text{Biological value (BV)} = \frac{N_2\text{retained}}{N_2\text{absorbed}} \times \frac{100}{1}$$

$$\text{Net protein ratio (NPR)} = \frac{\text{weight gain of treatment animal} - \text{weight loss of control animal}}{\text{Weight gain of treatment animal}}$$

Digestibility study

Towards the end of the experiment (starter and finisher phases: 4^{th} and 8^{th}weeks), two (2) birds per replicate will be randomly selected and placed on metabolic cages of wire floor type on the last three (3) days of the experiment, for the purpose of adaptation period, followed by four days of total collection of droppings. The total faeces voided during this period will be sundried and later oven dried; bulked and representative samples will be taken for chemical analysis according to A.O.A.C (1990). During the trial period, the quantities of feeds supplied to the birds will be recorded. In the nitrogen balance studies, nitrogen retention and apparent digestibility (AD) will be determined.

The apparent digestibility will be then calculated using the formula:

$$\frac{\text{Dry weight of diet eaten} - \text{Dry weight of faeces voided}}{\text{Dry weight of diet eaten}} \times \frac{100}{1}$$

Haematological study

Blood samples will be collected terminally from one bird per replicate at the end of the feeding trial (8weeks). About 5ml of blood will be collected from each bird by severing the jugular vein and blood allowed to flow freely into a labeled ethylene diamine tetra acetic acid (EDTA) containing specimen bottle for the estimation of haematological parameters. A second set of 5ml of blood will be also collected into heparinized bottles for serum chemistry.

Analytical procedure

Determination of haematological traits

Packed cell volume (PCV (%)

The anti –coagulant (EDTA) treated blood samples will be allowed to enter the capillary tube (leaving at least 15mm unfilled). The other end of the tube will be sealed by heating. The tubes will be then centrifuged for 10 minutes with a haemotocrit centrifuge at 10,000rpm, and PCV values read from a portable haematocrit reader. The results will be reported in percentages as described by Kelly (1980).

Red blood cell (RBC) count

In determining the total red blood cell count, anti-coagulant (EDTA) treated fresh blood samples will be diluted with 0.9% sodium chloride (NaCl) and well shaken according to Ojo (1999) and Dacie and Lewis (1991), this will be then mounted in a haemocytometer (Hawksley®, England) and the number of erythrocytes in a circumcised volume (0.02cubic millimitre) will be counted microscopically. The counted erythrocytes will be then expressed in millions per cubic millimiters.

White blood cell (WBC) count

White blood cell counts will be estimated by the haemocytometer method using the improved Hawskley haemocytometer as described by Dacie and Lewis (1991). The standard ratios of the mean corpuscular haemoglobin concentration (MCHC) will be calculated according to the method described by Jain (1986).The differential counts of the white blood cell will be determined by making a differential smear, using wrights strain, and the percentage lymphocytes, ensinophils, neutrophils and monocytes will be taken.

Haemoglobin concentration (Hb)

Twenty microlitres (20µl) of blood will be introduced into 4ml of diluents. The tube containing the solution will be covered and inverted several times, and allowed to stand at room temperature for 10minutes in order to allow for complete reaction leading to the formation of cyamenthyaemoglobin (HICN). The solution of cyamenthaemoglobin (HICN) will be read using a spectrophotometer at a wavelength of 540nm compared with a reagent blank standard (Dacie and Lewis, 1991).

Determination of serum constituents

Total protein, albumin, globuline, glucose, creatinine and urea will be determined using the method described by Dacie and Lewis (1991), while sodium (Na) and potassium (k) will be determined by flame photometry (Hawk, Oser and Sarmassen*,* 1954).

a. **Total protein:** Total protein will be determined by the burette method of Colowick and Kaplan (1955) as described by Dacie and Lewis (1991).

b. **Serum albumin and globulin:** Albumin and globulin will be determined by bromocresol method as described by Doumas and Briggs (1977).

c. **Serum creatinine and urea: S**erum creatinie will be determined by the alkaline picrate method of Bonsnes and Taussky (1945) and urea will be estimated by diaacetymonoxine method of Wootton (1964) as described by Dacie and Lewis (1991).

d. **Serum glucose:** Serum glucose will be determined using the Blotech Laboratory (1985) method.

e. **Serum Cholesterol:** Serum cholestearol will be determind as described by Hyduke (1975)

f. **Sodium and potassium:** Serum sodium and potassium will be determined using the flame photometry method of Hawk *et.al.* (1954).

Result and discussion

Performance characteristics

Results on the performance characteristics of broiler starter and finisher diets fed varied levels (0, 20, 40 and 60%) of African yam bean (AYB) based diets indicated that dietary treatments had significant ($P<0.05$) effects on the live weight and weight gains of chicken. Average final weight (starter) was highest in birds fed the control diet (0%AYB), followed by 20, 40 and 60%AYB respectively. The final weight of birds fed control and 20%AYB did not differ significantly ($P>0.05$) from each other at both starter and finisher phases.

Table 3: Growth performance of experimental broiler chickens fed starter diets (0-4 weeks).

	Diets				
	1	2	3	4	
	Percentage (%) levels of AYB inclusion as replacement for Soyabean meal protein				
	0	20	40	60	
Parameter					SEM±
Ave.initial weight (g/chick)	44.56	45.0	44.87	45.0	0.01
Ave.final weight (g/chick)	606.80[a]	586.68[ab]	558.11[b]	526.88[c]	2.45
Ave. weight gain ($g^{-\ day/chick}$)	20.08[a]	19.31[ab]	18.33[b]	17.21[c]	0.22
Ave. feed consumption ($g^{-\ day/chick}$).	44.5[ab]	44.9[a]	42.6[c]	41.7[c]	0.30
Feed convertion ratio	2.21	2.32	2.32	2.42	0.01
Protein efficiency ratio (%)	1.92[a]	1.84[b]	1.85[d]	1.77[c]	0.06
Mortality (%)	0.64	0.00	0.96	0.96	0.04

a-c: Means in the same row with varying superscripts differ significantly (P<0.05).
SEM±: Standard error of means

Table 4: Growth performance of experimental broiler chickens fed finisher diets (5-8 weeks).

	Diets				
	1	2	3	4	
	Percentage (%) levels of AYB inclusion as replacement for Soya bean meal protein.				
	0	20	40	60	
Parameters					SEM±
Ave.initial weight (g/chick)	606.80	586.68	58.11	526.88	2.45
Ave.final liveweight (g/chick)	1987.20[a]	1957.00[a]	1847.23[b]	1815.16[c]	319.0
Ave. daily weight gain (g – day/bird)	49.30[a]	48.94[a]	46.04[b]	46.01[c]	0.60
Ave. daily feed Consumption ($g^{-\ day/chick}$)	109.0	107.4	103.5	100.1	133.0
Feed convertion ratio	2.19[c]	2.19[c]	2.25[b]	2.18[a]	0.05
Protein efficiency ratio	2.17[a]	2.19[a]	2.14[b]	2.21[c]	0.04
Mortality (%)	--	--	--	--	--

a-c: Means in the same row with varying superscripts differ significantly (P<0.05).
SEM±: Standard error of means

At the starter stage, there were no significant (P>0.05) differences in the average weight gain of birds placed on 20%AYB and 40%AYB, while the birds in 20%AYB diet compared favourable with the control diet.The least weight gain was in birds fed 60% AYB. The findings in this study indicated that broilers fed starter diet containing up to 40% AYB seeds performed well without any adverse effect on the weight gain. At the finisher stage, significant (P>0.05) differences were also observed among birds fed test diets and growth decreased with increased levels of African yam bean in the diets. The results in this study agreed with the findings of Ojewola and longe (1999), Ani and Okeke (2003) and Emenalon (2004) who reported significant growth depression among birds placed on diets containing up to 20−50% cooked pigeon pea meal.

Average daily feed consumption of birds in all the dietary treatments at the starter stage were significantly (P<0.05) reduced with increasing levels of inclusion of AYB in the diets. However, the variation in feed consumption noticed at the starter phase was not observed on birds at the finisher phase. This could be ascribed to birds' ability for better digestion of cellulose. This feed consumption pattern of the chicks' reveals the positive correlation between feed intake and growth rate with corresponding increase in age. These trends suggest that broiler finishers were more adapted to intake of boiled AYB based diets. Onifade (1995) and Bolarinwa (1998) opined that for efficient growth rate in broilers and other monogastric animals, feed intake must correspondingly increase to meet the anticipated growth rate of animals. At the finisher phase, there were no significant (P<0.05) variations recorded in the average feed consumption of birds fed dietary treatments which could further explain the fact that AYB seeds contain considerable level of fibre. There were no significant (P>0.05) decrease in the feed convertion ratio of birds fed dietary treatments at the starter phase. Feed convertion ratio of bird's fed 0% AYB (control) suggest better feed utilization than those maintained on AYB diets.

At the finisher phase, no significant (P>0.05) variations were observed in feed convertion ratio in birds fed 0 and 20%AYB, but both were significantly (P<0.05) different from 40 and 60%AYB based diets. This shows that feed utilization significantly (P<0.05) varies within treatment means which agreed with the report by Oduguwa *et. al.* (2004) in the study of effect of enzymes supplementation on the utilization of strimp waste meal based diets by broiler chickens.

The protein efficiency ratio of broiler were significantly (P<0.05) influenced by dietary treatments. Better protein efficiencies were recorded as the quantity of AYB in the diets decreased at the starter stage and finisher phases. Mortality was recorded only at the starter phase, but without significant (P>0.05) effect, while none was observed at the finisher stage.

Haematological parameters

The haematological indices assessed in broiler chickens as influenced by varied levels of AYB in the diets are depicted in Table 5.

Results obtained showed that significant (P>0.05) differences were not observed among the values recorded for the experimental chickens. This is an indication of good nutritional status observed among the experimental birds.

Packed Cell Volume (PCV %) and Heamoglobin concentration (Hb).

The haemoglobin concentration of chickens were highest in the control 0% AYB, followed by 20%AYB, 40%AYB and 60%AYB (lowest) based diets respectively with corresponding values of 11.87, 11.77, 11.51 and 11.42g/dl. The packed cell volume (PCV %) of 31.43, 30.33, 30.29 and 31.45% were recorded for 0%AYB, 20%AYB, 40%AYB and 60%AYB based diets respectively.

According to Frandson (1981), a decrease in the quantity of haemoglobin below normal might be as a result of poor nutrition including dietary deficiencies of iron, copper, vitamins or amino acids. Harkbarth, *et.al.* (1983) stated that dietary influence in the haematological traits is very strong. The PCV and Hb concentrations according to Farinu (1984) have been shown to reveal the nutritional status of animals.

Table 5: Haematological paramerters of broiler chickens fed diets containing different levels of AYB inclusion as replacement for soya bean meal protein

	DIETS				
	1	2	3	4	
	Percentage (%) levels of AYB inclusion as replacement for Soya bean meal protein.				
	0	20	40	60	
Parameters					SEM±
Hb (g/dl)	11.87	11.77	11.51	11.42	0.16
PCV (%)	31.43	30.33	30.29	31.45	0.20
RBC($x10^{12}$/l)	3.75	3.55	3.54	3.66	0.22
WBC($x10^{12}$/l)	21.51	20.94	20.06	21.50	0.19
MCV (fl)	80.01	69.16	68.15	80.15	4.15
MCH (pg)	30.56	29.48	30.28	30.45	1.86
MCHC (g/l)	34.99	33.82	33.95	35.01	0.88

SEM±: Standard error of means

The Red blood cell (RBC) and White bloodcell (WBC):

The red blood cells (RBC) count was highest ($3.75x10^{12}$/l) in birds fed 0% AYB and least in birds fed 40%AYB ($3.54x10^{12}$/l). The white blood cells (WBC) count of 21.51x10, 20.94 x10, 20.60 x10 and 21.50 $x10^{12/}$l were obtained for birds fed diets containing of 0, 20, 40 and 60% AYB based diets respectively. Mean corpuscular volume (MCV) was highest (80.15fl) in birds fed 60%AYB, followed by birds fed 0%AYB (80.01fl), 20%AYB (69.16fl) and least in birds fed 40%AYB (68.15fl). Mean corpuscular haemoglobin (MCH) was highest (30.56pg) in the (control) 0%AYB, followed by 60%AYB (30.45pg), 40%AYB (30.28pg) and 20%AYB (29.48pg) based diets. Mean corpuscular haemoglobin concentration (MCHC) values of 34.99, 33.82, 33.95 and 35.01g/l were recorded for 0, 20, 40 and 60% AYB based diets respectively. The MCV, MCH and MCHC are readily used in the assessment of nutritional status of animals as a result of feed ingested.

Serum Constituent of broiler chickens

The parameters assayed in serum constituent of broilers namely total protein, albumin, globulin, creatinine and urea indicated no significant (P>0.05) difference as influenced by dietary treatments, except the serum cholesterol which varied significantly (P<0.05) as showed in Table 4.

Table 4: Serum constituent of broiler chickens

	Diets				
	0	2	3	4	
	Percentage (%) levels of AYB inclusion as replacement for Soyabean meal protein.				
	0	20	40	60	
Parameters					SEM±
Total protein (g/100ml)	7.35	7.40	7.25	7.20	0.11
Albumin (g/l)	3.40	3.41	3.43	3.45	0.15
Globulin (g/dl)	3.91	3.90	3.91	3.90	0.15
Creatinine (g/dl)	1.36	1.35	1.36	1.35	0.004
Urea (g/l)	19.45	18.46	18.36	18.34	0.16
Cholesterol (mg/100ml)	171.11^{a}	140.22^{b}	138.16^{bc}	122.16^{d}	1.20

a-d: Means in the same row with varying superscripts differ significantly (P<0.05).
SEM±: Standard error of means

Inclusion of AYB in the diet decreased. The values of 171.11, 140.22, 138.16 and 122.1mg/100ml were recorded for 0, 20, 40 and 60%AYB based diets respectively. This variation may be ascribed to the high level of oil in soya bean and invariably low level oil in AYB seeds. It is contrary and paramount to note the positive nutritional and health importance associated with AYB (i.e. arteriosclerosis and other cardiovascular disorder associated with hypercholesterosis) rather than the many hazards which were reported to be associated with the utilization of AYB in the past. This finding therefore agrees with that of Azeke, *et al.,* (2005).

Conclusion

In the broiler chicken experiment, different levels of (20, 40 and 60 %) boiled AYB seeds were incorporated in broiler diets as replacement for soya bean meal protein. The inclusion of AYB significantly (P<0.05) affected the performance characteristics indices of broilers. At the starter phase, significant (P<0.05) differences existed in weight gain, feed consumption and final weight of broiler chickens. At finisher phase, all others except daily feed intake, PER and mortality were significantly (P<0.05) affected by treatments effect. Feed convertion ratio and PER were more superior at both starter and finisher stages at 0% and 20% inclusion levels. All the haematological indices and the serum constituent of broiler indicated no significant (P>0.05) differences.

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