Research Journal of Poultry Sciences 3 (2): 27-31, 2010
ISSN: 1993-5285

Biochemical Evaluation of Serum Metabolites, Enzymes and Haematological Indices of Broiler-Chicks Fed with Varying Levels of Rumen Epithelial Scraps in Place of Fish Meal Proteins

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Abstract: The effect of various inclusion of rumen epithelial scraps as substitute for fish meal proteins on the feed intake, nitrogen retention, serum metabolites, enzymes and haematological parameters in broiler-chicks in their finisher phase were examined. Five set of meal with 0, 25, 50,75 and 100% inclusion of rumen epithelial scraps as substitute for fish meal proteins were formulated. About 100 days old chicks were fed a nutritionally sound starter mash for 4 weeks after which they were randomly divided into 5 groups and placed on the various formulated diets. The feed intake and the weight gain in each group was measured for the following 21 days after which the birds were sacrificed. Nitrogen retention, serum proteins, liver enzyme activities and haematological evaluation were carried out. The feed intake, nitrogen retention and the weight gain in broiler-chicks fed 75 and 100% RES inclusion diet were significantly ($p<0.05$) lower than those of broiler-chicks fed 0, 25 and 50% RES inclusion diet. The albumin/globulin ratio in broiler-chicks fed 100% RES inclusion diet was significantly ($p<0.05$) higher than those of broiler-chicks in other groups. Mean Cell Heamoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC) and Mean Cell Volume (MCV) in broiler-chicks fed 0, 25 and 75% RES inclusion diet were significantly ($p<0.05$) lower than those of broiler-chicks fed 50 and 100%, RES inclusion diet. However, neutrophils of broiler-chicks fed 0, 25 and 50% RES inclusion diets were significantly ($p<0.05$) higher than those of 75 and 100% RES inclusion diets. The Pack Cell Volume (PCV), Haemoglo bin Concentration (HBC), White Blood Cell (WBC), lymphocytes and monocytes compared favourably in all the groups. The liver alanine transaminase and aspartate transaminase acivities in all the groups examined compared favourably. It infered that the 25 and 50% RES inclusion as substitute for fish meal protein could be employed to maintain the quality of the broiler-chicks while minimizing the high cost of whole fish meal since RES is an abattoir by-product that is available locally.

Key words: Rumen epithelial scrap, fish meal, broiler-chicks, diet, abattoir, Nigeria

INTRODUCTION

A notable feed resource quite often used in feed formulation which requires urgent research attention for an alternative is fish meal due to its high cost (Agbede and Aletor, 2003). The high demand by man for fish and fish products places a restriction on the use of fish meal protein in livestock.

The major objective of poultry production is to produce meat and eggs efficiently at economical rate which is only possible by using cheaper locally available feed ingredient because feed alone constitutes 70-75% of the total cost of poultry production (Panda, 1989). Nutritionists have attempted to replace the fish meal protein with alternative source of protein. Akpodiete and Inoni (2000) used maggot meal and Fasuyi (2000) used cassava leaf protein concentrate to replace wholly or partially fish meal protein in broiler diet with significant success in relation to some growth indices. Agbede and Aletor (2003) also reported that fish meal protein in the diet for broiler-chicks could be replaced by 25% with Glyricidia leaf protein concentrate without adverse effect on performance in broiler-chicks.

The present trial seeks to evaluate the effects of the replacement of fish meal protein with varying levels of rumen epithelial scraps in the broiler-chick finisher diet on the nitrogen retention, serum metabolites, liver enzymes and haematological parameters of the broiler birds. Rumen epithelial scrap is an abattoir by-product which is rich in protein (about 53% crude protein) and has amino acids profile which in most cases is similar with those of fish meal.

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MATERIALS AND METHODS

Experimented protein source: The Rumen Epithelial Scra (RES) used in this study were obtained from the Bodija abattoir in Ibadan, Nigeria. It was sun dried, milled and sieved. Thereafter, the RES was used to formulate diets along with other ingredients purchased from Ebun Olu Farm, Km 9 Ikirun Road, Osogbo, Nigeria.

Experimental diets: About 5 iso-nitrogenous and iso-caloric diets were formulated with the basal as well as the proximate composition as shown in Table 1 and 2, respectively. Diet 1 was a control with 100% Fish Meal (FM). The fish meal protein was substituted at 25, 50, 75 or 100% with RES in diets 2, 3, 4 and 5, respectively.

Management of chicks and experimental layout: About 100 days old broiler-chicks (50 males+50 females) used for the experiment were electrically brooded at 37°C, at the Animal Science Research farm, University of Ibadan, Nigeria for the first 4 weeks and sexed as described by Laseinde and Oluyemi (1997) and later transferred to a metabolism cage for 3 weeks to allow the chicks acclimate to the experimental conditions.

During this period they were offered a 23 g kg^{-1} crude protein commercial broiler-starter mash (Guinea Feed) and water *ad libitum* such that no other variable was introduced. Completely Randomized Design (CRD) was adopted for the trial with a total of 5 experimental units. At the end of the acclimation period, the chicks were weighed and 10 males and 10 females (total = 20) were assigned to each of the five dietary treatments such that the group mean weights $diet^{-1}$ were identical (206.3±1.4 g). The broiler-chicks in their finisher phase were fed their respective experimental diets *ad libitum* for 21 days during which the daily feed consumption and group weight changes were measured. Faeces voided during the last five days were collected, weighed, dried at 55-60°C in an air-circulating oven for 72 h and preserved while the corresponding feed consumed was recorded.

Blood collection for analysis: At the end of the feeding trial, the chicks were starved overnight so as to empty the crop and the chicks weighed and sacrificed first by stunning followed by severing the jugular vein.

The blood was then allowed to flow freely into labelled bijour bottles one of which contained a speck of EDTA while the others were without EDTA. The blood in the EDTA-containing bijour bottles was processed for haematology while those in bottles without EDTA were processed for serum. The serum was kept deep frozen prior to analysis (Ogbu and Okechukwu, 2001).

Haematological and serum analyses: The Packed Cell Volume (PCV) was estimated by spinning about 75 Fl of each blood sample in heparinized capillary tubes in a haematocrit micro centrifuge for 5 min while the total Red Blood Cell Count (RBC) was determined using normal saline as the diluting fluid. The Haemoglobin Concentration (HBC) was estimated using cyanome-thaemoglobin method while the Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), the Mean Corpuscular Volume (MCV), the total protein, albumin and globulin of the serum were determined as described by Lamb (1981).

Table 1: Composition of experimental diets (%)

	Formulated diets (RES inclusion %)				
Ingredients	0	25	50	75	100
RES (53%CP)	-	9.21	18.40	27.60	36.80
FM (65%CP)	30.00	22.49	15.00	7.49	-
Cassava flour	36.80	35.10	33.40	31.71	30.00
Palm oil	5.00	5.00	5.00	5.00	5.00
Glucose	3.20	3.20	3.20	3.20	3.20
Sucrose	20.00	20.00	20.00	20.00	20.00
Cellulose	0.35	0.35	0.35	0.35	0.35
Bone meal	2.00	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00	1.00
*Vit./Min Pre-mix	0.65	0.65	0.65	0.65	0.65
Salt	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
ME(Kcal g^{-1})	2.85	2.85	2.85	2.85	2.85
Crude protein	19.50	19.50	19.50	19.50	19.50

*Contained vitamins A (4,000,000 iu); D (800,000 iu); E (14000 iu); K (760 mg); B (7.6 mg); Riboflavin (2800 mg); Pyridoxine (1520 mg); Thiamine (880 mg); D Pantothenic acid (4400 mg); nicotinic acid (18,000 mg); Folic acid (560 mg); Biotin (45.2 mg) and Trace elements as Cu (3200 mg); Mn (25600 mg); Zn (16,000 mg); Fe (12800 mg); Se (64 mg); (320 mg) and other items as Co (160 mg); Choline (190,000 mg); Methionine (20,000 mg); BHT (2,000 mg) and Spiramycin (2,000 mg) 1.0 kg^{-1}. RES: Rumen Epithelial Scrap

Table 2: Proximate composition (g/100 g DM)

	Formulated diets (RES inclusion %)				
Ingredients	0	25	50	75	100
Dry Matter (DM)	92.64	92.65	92.64	92.38	92.40
Proteins (crude)	19.82^{a}	19.82^{a}	19.80^{a}	19.32^{b}	19.21^{b}
Crude fibre	9.28^{a}	9.27^{a}	9.30^{a}	9.30^{a}	9.56^{b}
Crude fat	7.21^{a}	7.25^{a}	7.23^{a}	7.75^{b}	7.65^{b}
Ash	8.57	8.56	8.60	8.63	8.62
Nitrogen per extract	55.12	55.10	55.07	55.00	54.96

Enzyme assay: Alanine Transaminase (ALT) and Aspartate Transaminase (AST) were assayed using the method of Reitman and Frankel (1957).

Chemical analysis: The proximate composition of the ingredients, diets and faecal samples were determined by the method of AOAC (1990). Nitrogen retained was calculated as the algebraic difference between feed nitrogen and faecal nitrogen (on dry matter basis) for the period. The amino acid contents of the RES and the diets were analyzed by hydrolyzing the RESs (50-75 mg) for 24 h in a heating block previously heated at 110±1°C. The hydrolysate was cooled and quantitatively transferred to a -50 mL flask and diluted to volume with water. After

filtration, a 10 mL aliquot of the filtrate was heated in a rotary evaporator (40°C) to remove excess acid before analysis using High-Performance Liquid Chromatography (HPLC) with a Varian HPLC system (Palo Alto, CA) and a Shimadzu RF-535 Fluorescence detector (Tokyo Japan) set at an excitation wavelength of 325 nm and an emission wavelength of 465 nm. Separation was achieved in adsorbosphere OPA-HR (150×4.6 mm) column (Alltech, Carnforth, UK). The mobile phase was 1,4-dioxan and 2-propanol (HPLC grade). Methionine was determined as methionine sulfone and cysteine as cysteic acid after performic acid oxidation while tryptophan was determined as described by Miller (1967). To correct for slight fluctuations in amino acid peaks, DL-Amino-n-butyric acid was used as internal standard.

Statistical analysis: Data collected on the performance indices; haematology and total serum protein were presented as mean of 20 replicates±Standard Error of Mean (SEM). Analysis of variance was carried out. Level of statistical significance was taken at $p<0.05$ (Adamu and Johnson, 1997).

RESULTS AND DISCUSSION

The results of the amino acid composition of RES (Table 3) showed that it contained essential amino acids Lysine (0.87), Methionine (1.36), Threonine (28.47), Tryptophan (3.03), Isoleucine (4.38), Leucine (7.89), Valine (23.8), Arginine (1.67), Phenylalnine (25.53) and Histidine (24.34 mg g^{-1}).

The amino acid composition of the five diets compare favourably except in 75 and 100% RES Inclusion diets where Lysine, methionine and arginine were lower. The 75 and 100% RES inclusion diets were significantly ($p<0.05$) lower in crude protein and higher in crude fat. The 100% RES inclusion diet presented significant ($p<0.05$) higher crude fibre (Table 2). About 75 and 100% RES inclusion diets also presented significant ($p<0.05$) lower mean cell haemoglobin and neutrophil (Table 4). The aspartate and alanine transaminase activities compared favourably among all the treatments (Fig. 1 and 2).

The identical performance of chicks fed the 0, 25 and 50% RES inclusion diets might be due to the similarity of their dietary amino acids profiles (Table 3) except for methione that was lower in 25% RES inclusion diet. There was a decrease in the concentration of some of the amino acids in the diets as the levels of RES substitution increased. This was more obvious in lysine, methionine, isoleucine and arginine (Table 3).

Thus, the observed decline in the average feed intake, average weight gain and nitrogen retention of the chicks fed 75 and 100% inclusion diets may be attributed in part to the adverse effect of amino acid imbalances. This corroborated an earlier report of Agbede and Aletor (1997) that amino acids imbalance adversely impair appetite and feed intake with attendant reduction in performance in chicks.

The crude fat of the 75 and 100% RES inclusion diets was significantly ($p<0.05$) higher than those of the control, 25 and 50% RES inclusion diets. Similar results were obtained by Agbede and Aletor (2003) for fish meal protein replaced by varying levels of Glyricidia leaf protein concentrate. This was attributed to the observed high fat deposition around the pericardium of the heart

Table 3: Amino acid composition of the diets (mg g^{-1})

Essential amino acids	Precentage of RES inclusion				
	0	25	50	75	100
Lysine	14.3	14.50	19.70	3.23	0.87
Methionine	5.4	2.02	3.98	1.46	1.36
Threonine	27.9	30.00	29.60	28.30	28.47
Tryptophan	7.5	7.40	7.00	3.30	3.03
Isoleucine	10.9	23.40	14.50	9.50	4.38
Leucine	19.1	17.50	16.70	12.40	7.89
Valine	27.9	28.70	30.50	25.30	23.80
Arginine	22.1	21.40	19.60	10.30	1.67
Phenylalanine	23.7	23.80	24.50	24.80	25.53
Histidine	12.7	12.80	16.40	18.80	24.34

Table 4: Haematological indices of broiler-finisher fed varying dietary levels of RESID

Diet	Percentage of RES inclusion				
	0	25	50	75	100
PCV (%)	45.1±1.5	45.2±2.3	44.9±3.2	44.5±1.2	44.80±2.7
RBC (×10^6 mm^{-3})	3.8±0.0	3.9±0.6	3.8±0.2	3.8±0.2	3.85±0.4
HBC (g/100 mL)	9.8±0.2	9.7±1.2	9.6±1.6	9.0±0.2	8.50±0.5
MCHC(%)	5.3±0.5^a	5.6±3.4^a	5.9±6.1^{ab}	5.3±0.9^a	6.60±1.1^b
MCH (pg)	21.7±1.2^a	20.6±2.2^a	19.5±7.1^b	19.7±1.2^b	19.00±0.7^b
WBC (×10^3 mL^{-1})	11.2±1.8	11.1±1.5	11.2±1.2	11.0±1.0	10.90±1.6
Neutrophil (%)	68.0±1.3^a	68.1± 1.2^a	67.6±1.3^a	64.5±1.0^b	63.00±1.1^b
Lymphocytes (%)	25.0±1.2	24.5±1.4	24.6±1.1	21.6±1.2	20.00±1.6
Monocytes (%)	6.0±1.3	6.5±0.9	6.2±1.1	5.9±1.2	8.00±1.0
MCV (µm^3)	121.1±2.2^b	117.0±1.9^{bc}	135.6±2.7^a	113.9±1.1^d	116.60±1.6^c

Means with differing superscript in the same row are significantly different ($p<0.05$). PCV = Packed Cell Volume; RBC = Red Blood Cell; WBC = White Blood Cell; HBC = Haemoglobin Concentration; MCHC = Mean Cell Haemoglobin Concentration; MCH = Mean Cell Haemoglobin; MCV = Mean Cell Volume, RESID = Rumen Epithelial Scrap Inclusion Diet

and implicated for the dystolic and systolic systems of the heart of the chicks fed on the test diets. Despite this, the absolute weight gain of the chicks fed 75 and 100% RES inclusion diets were lower than those fed the control, 25

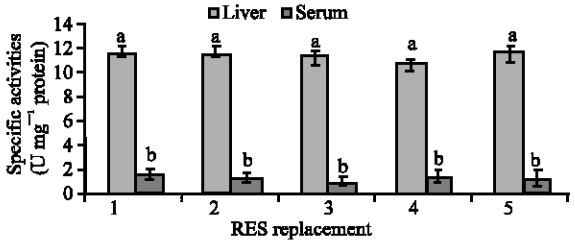

Fig. 1: Specific activities of alanine transaminase in the broiler-chicks fed the formulated diets. Each value is a mean of twenty determination±SEM. Bars with different letters are significantly ($p<0.05$) different. 1, 2, 3, 4 and 5 are 0, 25, 50, 75 and 100% RES replacement for fish meal protein, respectively

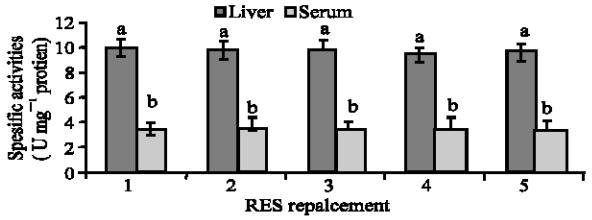

Fig. 2: Specific activities of aspartate transaminase in the broiler-chicks fed the formulated diets. Each value is a mean of twenty determination±SEM. Bars with different letters are significantly ($p<0.05$) different. 1, 2, 3, 4 and 5 are 0, 25, 50, 75 and 100% RES replacement for fish meal protein, respectively

and 50% RES inclusion diets (Table 5) thus further confirming the superiority of FM and FM protein replaced at 25 and 50% with RES as good sources of protein for broiler-chicks. Blood represents a means of assessing clinical and nutritional health status of animals in feeding trial and the haematological parameters (Table 6) most commonly used in nutritional studies include PCV, RBC, HBC, MCHC, MCV and clotting time (Aletor and Egberongbe, 1992; Olorode and Longe, 2000; Adeyemi *et al.*, 2000). The results of haematological variables in this study suggest that the test diets did not precipitate much severe effects on the health status of the experimental chicks.

However; the MCH values in broiler-chicks fed 0 and 25% RES inclusion diets were significantly ($p<0.05$) lower than those of broiler-chicks fed 50, 75 and 100% RES inclusion diet. These values were generally higher than those obtained by Agbede and Aletor (2003) for broiler starters fed fish meal replaced by varying levels of leaf protein concentrate from Glyricidia.

Although, total serum protein may be used as an indirect measurement of dietary protein quality (Tewe, 1985), the values observed for the serum generally compare favourably. However, the albumin/globulin ratio for broiler-chicks fed 75% RES inclusion diet was significantly ($p<0.05$) higher than those of broiler-chicks fed 0, 25, 50 and 100% RES inclusion diets.

The activities of enzymes such as GOT, GPT and GGT could be important in the diagnosis of diseases as well as in the investigation and thorough assessment of feed, drugs and extracts used in the treatment as these could give indications of progressive toxicity long before the actual manifestation of the toxic effects (Hanley *et al.*,

Table 5: Performance and nitrogen utilization of the broiler-chicks fed experimental diets (RESID)

	Percentage of RES inclusion				
Diet	0	25	50	75	100
Initial weight (g)	206.9±4.00	208.3±4.20	204.50±0.20	206.2±4.2	205.8±3.40
Final weight (g)	686.2±14.6^{a}	645.3±32.7^{a}	593.00±33.4^{b}	508.4+5.8^{c}	459.5±15.2^{c}
Average weight gain (g/chick/day)	22.0±0.70^{a}	20.8±1.60^{ab}	18.50±1.60^{b}	14.4±1.2^{c}	12.1±0.70^{c}
Average feed consumption (g/chick/day)	43.7±1.70^{a}	46.8±5.30^{a}	42.90±4.70^{a}	38.1±1.4^{b}	34.1±1.90^{b}
Feed efficiency	2.0±0.10^{c}	2.3±0.20^{b}	2.32±0.10^{b}	2.6±0.1^{a}	2.8±0.20^{a}
N-retention (g/chick/day)	0.9±0.30^{a}	0.8±0.30^{ab}	0.8±0.400^{ab}	0.7±0.2^{b}	0.6±0.30^{c}

Means with differing superscripts in the same row are significantly different ($p<0.05$)

Table 6: Serum metabolites of broiler-finisher fed varying dietary levels of RES

Diets (Percentage of RES)	Total protein (g/100 mL)	Serum albumin (g/100 mL)	Globulin (g/100 mL)	Albumin/Globulin ratio
0	6.96±0.3	2.70±0.4	4.26±0.9	0.63±0.2^{b}
25	6.7±0.30	2.5±0.10	4.2±0.30	0.60±0.1^{b}
50	6.6±0.40	1.9±0.50	4.7±0.50	0.40±1.0^{a}
75	6.0±0.40	2.1±0.20	3.9±0.20	0.54±0.1^{ab}
100	6.23±0.3	2.73±0.3	3.50±0.3	0.78±0.2^{c}

Mean with different superscript in the same column are significantly different ($p<0.05$)

1986). The results of alanine transaminase and aspartate transaminase in the liver and the serum showed that the control and the test diets did not precipitate any significant harmful effect on the health status of the liver of the broiler-chicks.

CONCLUSION

The results for the various RES inclusion as replacement for fish meal protein showed that fish meal protein replaced at 25 and 50% with RES could performed well as broiler-chicks diet in the finisher phase without adverse effects on the feed intake, average life weight gain, nitrogen retention, serum protein, haematological indices and liver enzymes of the broiler birds. This will reduce the high cost of procuring a whole fish meal since RES is an abattoir by-product that is locally available.

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