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*Original Article*

## Haematological and hepatic indices of cockerels fed treated dietary *Blighia sapida* seeds

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**ABSTRACT:** Ninety-six day old Lairier cockerel chicks were used in an experiment to evaluate the after effects of detoxifying (soaking, boiling, addition of riboflavin and glycine to antagonize hypoglycins) dietary *Blighia sapida* (ackee apple) seed meal, BSSM. Blood chemistry, haematology, liver morphology indices which dietary BSSM influenced and reflected in the performance characteristics of the cockerels investigated in a single-factor experimental design experiment were assessed. Results showed that the residual phytotoxins of BSSM, hypoglycins A & B with their metabolite MCPA at 17.50% inclusion of the processed BSSM in diets elicited reduction in glucose, protein, albumin, globulin while elevating blood cholesterol, creatinine, urea, total and conjugated bilirubin relative to the reference diet ( $p < 0.05$ ). Dietary BSSM similarly increased the transaminase activities of AST/SGOT, ALT/SGPT including ACP ( $p < 0.05$ ). BSSM based diets also caused significant reduction in PCV, RBC, WBC, Hb as well as MCV, MCH and MCHC similar to the results obtained on WBC differential counts of neutrophils and lymphocytes in comparison with the conventional diet ( $p < 0.05$ ). Histopathological examinations on the liver samples revealed that the control diet presented livers that were normal in tissue morphology without inflammation or haemorrhage while the photomicrographs of the liver samples of cockerels fed treated dietary BSSM at 17.50% inclusion showed morphological patterns indicating severe distortion suggesting evidence of haemorrhage and inflammation with numerous blood cells occupying the available hepatic sinuses. The poor results recorded on the biochemical, haematological and morphological parameters were reflected in performance characteristics as reduced feed intake, weight gain, growth rate, feed efficiency and high mortality were obtained on diets containing BSSM compared with the orthodox diet ( $p < 0.05$ ). Findings of this experiment indicated that for optimum results, processed BSSM be included in diets below the 17.50% level considered high for the birds in this study.

**KEYWORDS:** Cockerels, BSSM, treatments, blood chemistry, haematological and performance indices

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### INTRODUCTION

Plants on which human and animal life depends for food contain thousands of phytochemicals or allelochemicals (Rosenthal et al. 1979) which constitute antinutritional or beneficial factors to the consumer. The antinutrients implicated in limiting the availability or utilization of nutrients in *Blighia sapida* (commonly called ackee apple) are hypoglycin A (HGA) and hypoglycin B (HGB). Hypoglycins are part of a large family of oligopeptides and other amino acid derivatives produced in plants. HGA is a non-proteinogenic amino acid (L-alpha-amino-beta-methylene cyclopropane propionic acid), and its B derivative is gamma-L-glutamyl-a-amino-B-(2-methylene cyclopropyl) propionic acid (Kean and Hare, 1980). HGA is metabolized by transamination and oxidative decarboxylation to methylene cyclopropyl acetic acid (MCPA). Both hypoglycins and their metabolite are toxic to humans causing vomiting and coma (Scott, 1917; Jeliffe and Stuart, 1954; Meda, 1999) and death within 12

hours of ingestion in severe cases (Hill, 1953; Hassal and Hill, 1955; Brown et al. 1992). The vomiting sickness caused by hypoglycins is called Jamaica vomiting sickness (JVS). The condition is also known as Toxic Hypoglycemic Syndrome (THS).

Ingestion of 12 and 24 raw ackee fruits by adult humans is known to cause vomiting, drowsiness hypoglycemia and coma (Golden et al., 1984). Experimental analyses on animals showed that HGA caused fatty degeneration of the liver (van Holt and von Holt, 1959). Research on *Blighia sapida* extracts (Michael et al., 1998) revealed that neutrophil and platelet counts were significantly lowered in mice treated with aqueous and lipid extracts of *Blighia sapida*. This study is designed to examine some biochemical, haematological aspects and liver morphology of cockerels fed processed *Blighia sapida* seed meal (BSSM) in diets. Data obtained will be used as toxicity parameters to measure cellular and cell components of the cockerels given the control diet compared with changes in composition of the groups fed the test feedstuff in diets.

The range of variations observed will be used to assess the pathological and metabolic disorders and the adverse effects on the liver morphology in the fed cockerels.

## MATERIALS AND METHODS

### Material preparation

*Blighia sapida* seeds obtained from ripened fruits harvested from trees grown in Ilorin were used for the study. After they were separated from the fruits, the seeds were sun-dried to ease crushing with mortar and pestle. Crushed samples were soaked (48 hours) and boiled (90 minutes) to leach out some of the hypoglycins. Following each treatment, soaked or boiled water was decanted to eliminate the leached chemicals in solution. The dough after the treatments was sun-dried, and grinded using an attrition miller before storing in a polyethylene bag for subsequent inclusion in diet mixtures.

### Diets formulation, animals and feeding trial.

Four treatment diets containing identical energy and protein values to meet day old chicks requirement were formulated (NRC, 1994). The composition of the experimental diets on as fed basis is presented on Table 1. The control diet, 1 was made of maize and soybeans as the basic ingredients while diets 2, 3 and 4 contained 17.50% treated BSSM to which hypoglycins agonists, glycine and riboflavin were added at 0.75%, 1.00% and 1.25% respectively. Administration of riboflavin and glycine to antagonize the hypoglycins intoxication was suggested by Duff et al. (1980) on the basis that riboflavin could stimulate the synthesis of acyl-CoA dehydrogenases which are inactivated by the hypoglycins and the metabolite; while glycine could bind the excess dicarboxylic acids produced due to impaired lipid metabolism causing the corresponding sicknesses (Al-Bassam and Sherratt, 1981).

**TABLE 1** Percent composition of the experimental diets on as fed basis

Ingredients	Treatments			
	1	2	3	4
Maize	54.75	46.50	49.00	50.00
Soybean meal	35.50	30.50	24.00	18.50
BSSM	0.00	17.50	17.50	17.50
Bone meal	1.50	1.50	1.50	1.50
Oyster shell	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
DL-methionine	0.25	0.25	0.25	0.25
Vit.min.premix	0.50	0.50	0.50	0.50
Palm oil	6.25	2.50	4.00	7.50
Glycine	0.00	0.75	1.00	1.25
Riboflavin	0.00	0.75	1.00	1.25
Lysine	0.25	0.25	0.25	0.25

Ninety six (96) day-old cockerel chicks (Lairier breed) were used for the research. They were housed in an electrically heated battery brooder cage with bulbs installed to provide heat for the brooding period to obtain a brooding temperature of 32-35 °C (Oluyemi and Robert, 1979). Each dietary treatment had 24 chicks with 8 chicks per replicate. The experiment was designed as a one-way classification. Chicks were given feed and drinking water twice daily, 08.00 hours and 15.00 hours. Both feed and water were supplied to appetite during the duration of the experiment. In the course of the trial, data were collected on feed intake, weight gain, growth rate, efficiency of feed utilization, survival rate and phenotypic observations. At the end of the feeding trial, one cockerel per replicate on all the treatments was randomly taken and sacrificed. Evisceration of the liver samples was accomplished and they were preserved in neutral buffer formalin (4% formaldehyde in phosphate buffered saline) for subsequent histopathological and morphological studies. Whole blood and sera samples were taken from the replicate cockerels slaughtered for the analyses of serum biochemistry and haematological determinants. Whole blood samples were collected in EDTA treated bottles to avoid clotting for the analyses of haematological indices, while sera samples for serological examination were allowed to stand for sometime in test tubes and further centrifuged to obtain clear sera prior to analyses.

### Chemical analyses

Haematocrit (PCV) was determined by spinning about 75 µl of each blood sample in capillary tubes in a haematocrit centrifuge for 5 minutes. Erythrocytes (RBC counts) was estimated using normal saline as the diluting fluid while leucocytes (WBC counts) were determined by employing glacial acetic acid, distilled water, 1% aqueous solution of gentian violet and 0.1N HCl as diluting fluid. Haemoglobin (Hb) concentration was estimated using the method described by Swensen (1951). Blood glucose, albumin, globulin and total protein were determined according to the method of Lamb (1981). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as described by Jones and Hunt (1983). Blood urea nitrogen, cholesterol and creatinine values were determined as outlined by Sirois (1995). The activities of the serum enzymes, alanine aminotransferase (GPT), aspartate aminotransferase (GOT), alkaline and acid phosphatases (ALP & ACP) were analyzed using the colourimetric method of Sigma Diagnostic (1985). Morphological or histopathological examinations on the liver samples were carried out as described by Nestle (1993).

### Statistical analyses

Data obtained on serum biochemistry, haematology and performance characteristics were analyzed by ANOVA using the model for a single-factor design (Steel et al. 1997). Differences among treatment means were compared by using the least significant test at 5% probability level (Gomez and Gomez, 1984). All data are reported as means and analyzed except those on basophils, eosinophils and monocytes which could not form ANOVA procedures and were subjected to descriptive statistics.

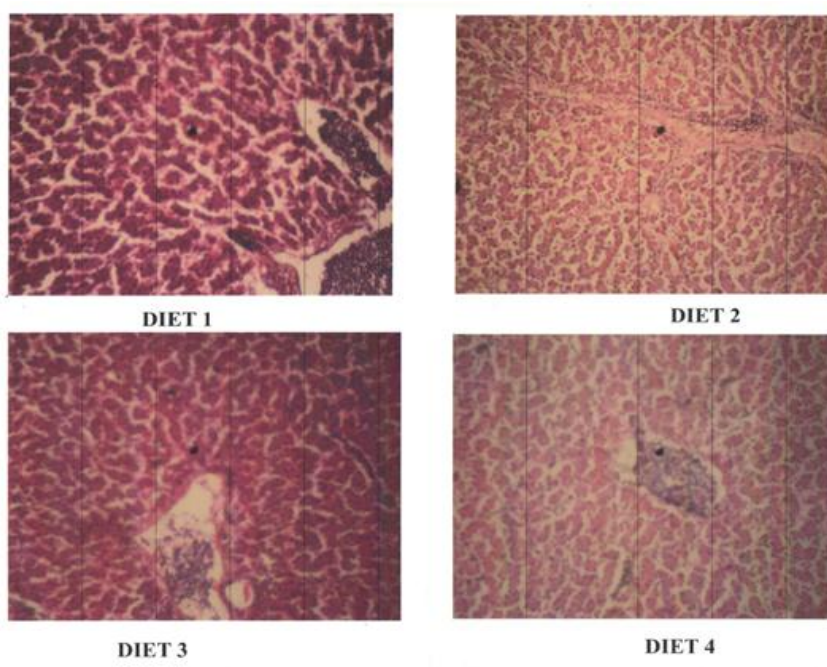


FIGURE 1 The photomicrographs of livers on BSSM diets compared with the control

RESULTS

The blood chemistry indices of cockerels fed treated dietary BSSM is shown in Table 2. BSSM based diets caused a significant decrease in glucose level, total protein, albumin and globulin ( $p < 0.05$ ), while increasing blood cholesterol, creatinine, urea, total and conjugated bilirubin relative to the control diet ( $p < 0.05$ ). Processed dietary BSSM increased the activities of AST, ALT and ACP in comparison with the activities of the group of birds maintained on the reference diet ( $p < 0.05$ ). The histopathological examinations of the liver samples are shown in Figure 1 (micrographs 1-4). The photomicrographs of the liver on the reference diet 1 presents normal hepatic tissue morphology without any inflammation or haemorrhages. Micrographs 2-4 of cockerels given processed BSSM diets revealed morphological patterns showing distortion of the liver architecture with respect to hepatic cell morphology and also evidence of haemorrhage and inflammation of the hepatic cells.

Ingestion of the treated BSSM included at 17.50% in diets significantly decreased PCV, RBC, WBC and HB compared with the values on the conventional diet ( $p < 0.05$ ). The test diets similarly lowered the MCV, MCH, MCHC as well as the WBC differential counts on neutrophils and lymphocytes in comparison with results on the standard diet ( $p < 0.05$ ). Few or no cell counts were obtained on basophils, eosinophils and monocytes and could not be statistically analyzed hence followed descriptive statistics (Table 3). Table 4 shows data on performance characteristics of the experimental birds receiving BSSM diets compared to the control ration. Consumption of treated dietary BSSM resulted in weight loss, negative growth and efficiency of feed utilization while producing high mortality on the diets with 17.50% BSSM ( $p < 0.05$ ).

TABLE 2 Blood chemistry indices of cockerels fed treated BSSM diets relative to the control diet

Diets indices	1	2	3	4	SEM
Glucose level, mmol/l	2.27 <sup>b</sup>	2.27 <sup>b</sup>	1.70 <sup>a</sup>	1.57 <sup>a</sup>	0.04
Total protein, mmol/l	40.00 <sup>c</sup>	36.67 <sup>b</sup>	34.00 <sup>b</sup>	22.00 <sup>a</sup>	0.56
Albumin, mmol/l	22.67 <sup>b</sup>	20.33 <sup>b</sup>	17.67 <sup>a</sup>	16.67 <sup>a</sup>	0.82
Globulin, mmol/l	15.67 <sup>c</sup>	12.67 <sup>b</sup>	10.67 <sup>a</sup>	10.00 <sup>a</sup>	0.92
Cholesterol, mmol/l	0.82 <sup>a</sup>	1.01 <sup>b</sup>	1.27 <sup>b</sup>	1.59 <sup>b</sup>	0.01
Creatinine, mmol/l	31.00 <sup>a</sup>	34.00 <sup>b</sup>	34.67 <sup>b</sup>	40.00 <sup>c</sup>	2.86
Urea, mmol/l	3.40 <sup>a</sup>	3.87 <sup>a</sup>	4.17 <sup>b</sup>	4.59 <sup>b</sup>	0.02
Total bilirubin, mmol/l	2.60 <sup>a</sup>	12.10 <sup>b</sup>	15.30 <sup>b</sup>	18.60 <sup>c</sup>	
Conjugated bilirubin, mmol/l	1.80 <sup>a</sup>	6.00 <sup>b</sup>	6.10 <sup>b</sup>	7.80 <sup>b</sup>	
AST (SGOT), IU/L	90.00 <sup>a</sup>	110.00 <sup>a</sup>	127.00 <sup>b</sup>	128.00 <sup>b</sup>	2.67
ALT (SGPT), IU/L	40.00 <sup>a</sup>	71.00 <sup>c</sup>	75.00 <sup>d</sup>	65.00 <sup>b</sup>	4.20
ALP (IU/L)	131.00	145.00	139.00	131.00 NS	2.67
ACP (IU/L)	5.23 <sup>a</sup>	5.43 <sup>a</sup>	6.60 <sup>b</sup>	7.73 <sup>c</sup>	2.67

Means without common superscripts (a,b,c,d) on the same row are significantly different ( $p < 0.05$ )

**TABLE 3** Haematological parameters of cockerels given processed BSSM based diets

Diets parameters	Treatments				SEM
	1	2	3	4	
PCV (%)	25.00 <sup>c</sup>	21.66 <sup>b</sup>	18.66 <sup>a</sup>	16.00 <sup>a</sup>	2.96
RBC ( $\times 10^{12}/l$ )	2.57 <sup>c</sup>	2.32 <sup>c</sup>	2.08 <sup>b</sup>	1.89 <sup>a</sup>	7.05
WBC ( $\times 10^9/l$ )	70.73 <sup>c</sup>	68.00 <sup>c</sup>	62.00 <sup>b</sup>	36.53 <sup>a</sup>	3.99
Hb (g/dl)	7.60 <sup>b</sup>	6.63 <sup>a</sup>	5.63 <sup>a</sup>	5.03 <sup>a</sup>	1.72
MCV (pg)	111.00 <sup>c</sup>	96.00 <sup>ab</sup>	88.66 <sup>ab</sup>	84.33 <sup>a</sup>	1.65
MCH (fl)	28.66 <sup>c</sup>	26.33 <sup>c</sup>	25.00 <sup>b</sup>	23.66 <sup>a</sup>	7.37
MCHC (%)	31.00 <sup>b</sup>	30.00 <sup>b</sup>	29.00 <sup>a</sup>	26.00 <sup>a</sup>	8.32
Neutrophils, %	24.00 <sup>c</sup>	18.00 <sup>b</sup>	15.33 <sup>b</sup>	12.00 <sup>a</sup>	2.07
%Lymphocytes	86.00 <sup>c</sup>	82.00 <sup>c</sup>	78.00 <sup>b</sup>	70.00 <sup>a</sup>	1.44
Eosinophils, %	2.33	1.50	2.00	1.00	-
Monocytes, %	1.00	-	-	1.00	-
Basophils, %	2.00	-	1.00	-	-

Means without common letters (a,b,c) on the same row are significantly different ( $p < 0.05$ )

**TABLE 4** Performance characteristics of cockerels maintained on treated dietary BSSM

Diets response criteria	1	2	3	4	SEM
Feed intake, g/b/d	90.00 <sup>c</sup>	45.00 <sup>b</sup>	42.00 <sup>b</sup>	34.00 <sup>a</sup>	0.33
Weight, g/b/d	16.30 <sup>a</sup>	-38.00 <sup>b</sup>	-168.00 <sup>c</sup>	-240.00 <sup>d</sup>	1.49
Growth rate, g/b/d	2.43 <sup>a</sup>	7.32 <sup>b</sup>	21.51 <sup>c</sup>	25.38 <sup>d</sup>	0.14
Feed efficiency	0.18 <sup>a</sup>	-0.84 <sup>b</sup>	-3.90 <sup>c</sup>	-2.31 <sup>c</sup>	0.05
Mortality rate, %	20.83	54.20	75.00	83.33	

a-b-c-d means in rows not sharing common superscripts differed significantly ( $p < 0.05$ ).

## DISCUSSION

Dietary BSSM caused significant reduction in serum glucose level. Decrease in blood glucose which measures the sugar level in the blood as found in this study could be attributed to the toxic hypoglycaemic effects of the residual hypoglycins and their metabolite, MCPA, produced *in vivo* by transamination and oxidative decarboxylation. Findings on depletion of blood glucose

level, total protein, albumin and globulin observed in this work agreed with those of published studies (Bressler et al. 1969; Tanaka et al. 1976; Mills et al. 1987; AMA, 2004) which reported similar adverse effects of ackee phytotoxins following biochemical investigations. Decrease in total protein and albumin in the cockerels suggest hypoproteinaemia while decrease in globulins, the antibody proteins important for fighting diseases in the body indicate that the birds' ability to fight diseases attack is reduced. The increase in blood cholesterol level on BSSM based diets as well as the increase in the levels of creatinine, urea, total and conjugated bilirubin could be indicative of hypertension in the fed cockerels. Elevated cholesterol level and the toxic effects of hypoglycins and its metabolite on the other serum biochemical indices and haematological metabolites of total and conjugated bilirubin could be indications of toxicity at significant high levels.

Serum enzymes such as AST, ALT, ACP are used in the estimation of liver function thus the significant increase in the activities of the mentioned marker enzymes in the cockerels fed 17.50% BSSM diets may signify hepatitis. Histopathological examination of the hepatic tissues indicated the presence of distortion of the liver architecture with regards to hepatic cell morphology and also evidence of haemorrhage and inflammation of the cells suggesting the negative influence of the residual BSSM phytotoxins. Such deleterious effects could adversely interfere with the organ functions which include protein synthesis, production of biochemical substances necessary for digestion and detoxification.

Ingestion of processed BSSM in diets decreased PCV, RBC, WBC, Hb as well as MCV, MCH, MCHC including the WBC differential counts on neutrophils and lymphocytes. Results on the haematological parameters are in consonant with the reports of Harper et al. (1979) which stated that ingestion of numerous dietary compounds is attended with measurable adverse effects on blood constituents. Results on WBC differential counts in this experiment agreed with the findings of Michael et al. (1998) who noted that the differential and platelet counts in mice were significantly lowered following treatment with aqueous and lipid extracts of *Blighia sapida*. The presence of few cell count on eosinophils, monocytes, basophils or their total absence following intake of dietary BSSM may suggest that the non-granular monocytes, which usually change into macrophages to destroy germs might not be adequate or available to perform their functions (Dacie and Lewis, 1977; Agrawal and Mahagan, 1980; Jain, 1986).

Performance data indicated that including treated BSSM in diets at 17.50% failed to improve or support feed consumption, weight gain and growth rate which were reflected in poor result on efficiency of feed utilization. Poor results on performance were further ascertained by the high mortality rate incurred on diets containing BSSM confirming early works (Addae and Melville, 1988; CDC, 1992; Larson et al. 1994; McTague and Forney, 1994; Moya, 2001; Eddlestone et al. 2003; Joskow et al. 2006).

In summary, results of this experiment show that although BSSM was processed before inclusion in diets, the residual effects of ackee phytotoxins probably due to administration at 17.50%



considered high for the cockerels might have produced the observed poor results. Hence for optimum utilization levels below 17.50% are recommended.

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