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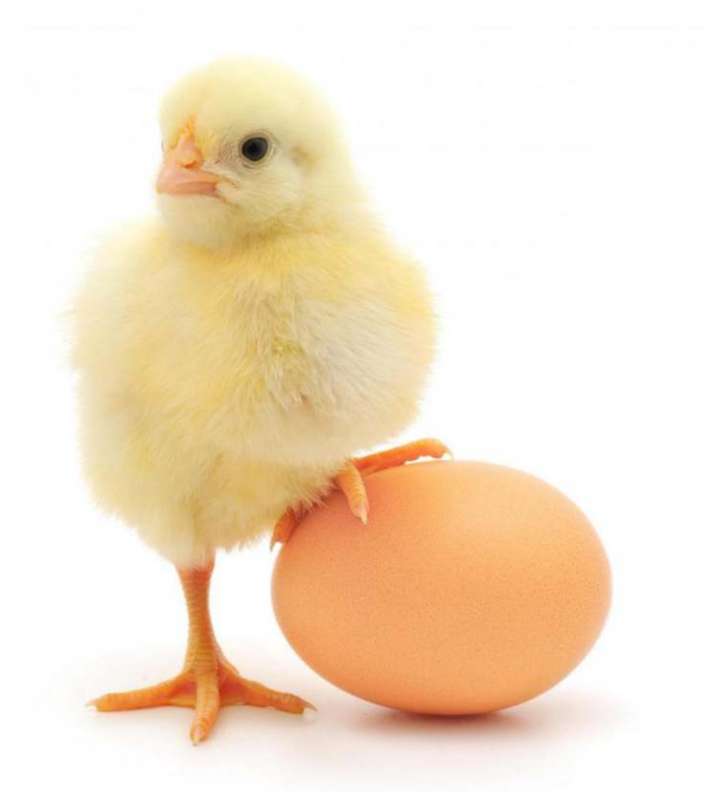

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Glycine and Riboflavin Detoxification of Ackee Apple Seeds *(Blighia sapida)*: Effects on Blood, Git and Organ Development in Broilers

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Abstract: Ackee Apple Seeds (AAS) subjected to chemical detoxification with glycine and riboflavin at 5% coupled with traditional treatments of soaking and boiling to leach out some soluble AAS toxins, hypoglycin A and B, was evaluated for nutritional adequacy using day chicks (DOC). Six iso-caloric and nitrogenous diets were prepared with graded levels of 2.5, 5.0, 7.5, 10.0 and 12.5% AAS meal in rations 2, 3, 4, 5 and 6 respectively. The diets including a corn-soybeans control diet were fed *ad libitum* to 144-DOC in a 4-week feeding trial. Results showed that the test feedstuff improved blood composition (PCV, WBC). The non-significant difference recorded on lengths of GIT parts including ileum and duodenum relative to the reference diet suggest the similarity of the test diets with the conventional diet ($p>0.05$). Differences observed on absolute and relative organ weights (AOW and ROW) supported the result on performance which gave significant increase in body weight gain ($p<0.05$). Results on hematology, GIT, AOW and ROW following detoxified AAS meal ingestion suggest that the methods offer great potential for effective detoxification of Ackee apple seeds.

Key words: Ackee apple seeds, glycine, riboflavin

INTRODUCTION

The need to detoxify oil seeds arise from the adverse effects of toxins and anti-nutritional factors on the nutritional quality of the seeds to the fed livestock. Various methods of detoxification are available depending on the toxins or anti-nutrients in seeds and its influence. Chemical treatment with glycine and riboflavin employed in this study are therefore some of the methods of detoxification.

Glycine and riboflavine are considered (Al-Bassam and Sherratt, 1981) because preliminary studies using chemicals to detoxify ackee apple seed in poultry nutrition (Kadri and Annongu, 2009; Gawati and Annongu, 2010) failed to yield positive results as the experimental birds died within a week of the trial lasting for 4-weeks. Earlier work to detoxify ackee apple fruits by traditional methods of soaking and/or boiling (Morton, 1987) were unable to remove the toxins but could only reduce the concentration by leaching.

Ackee apple (*Blighia sapida*) contains several phytotoxins which hinder the nutritional utilization of the leaves, fruits, seeds or arilli. Notable among the toxins are two toxic compounds, hypoglycins A and B because of their hypoglycemic activity. Hypoglycin is a water soluble natural toxin similar to leucine and iso-leucine in chemical structure, chromatographic and photometric properties (Riffle and Robert, 1998). Other metabolites isolated from ackee apple fruits though not as biologically active as hypoglycins include blighione, vomifoliol, glycine (Stuart *et al*., 1976).

It is sequel to the toxic nature of the fruits that attempts were made to detoxify the seeds for use as an alternative protein feedstuff for poultry in place of the conventional soybean protein which is a competitive plant protein between humans and animals in developing and underdeveloped countries.

MATERIALS AND METHODS

Ackee apple seeds were obtained from matured fruits from trees grown in Ilorin environs. Seeds were sun-dried before crushing in a mortar to increase the surface area for effective leaching of some toxins. After a 72-h soaking, water was decanted and the dough immersed in boiling water for 90-min before the water was drained to enable speedy drying. The dried material was grinded into meal with an attrition miller prior to incorporation in diet mixtures. Six dietary treatments were formulated made of a maize-soybeans control diet and diets 2, 3, 4, 5 and 6 containing processed Ackee Apple Seed Meal (AASM) inclusion of 2.50, 5.00, 7.50, 10.00 and 12.50% treated with glycine and riboflavin respectively. 144-day old broiler chicks used for the experiment were housed in an electrically heated metabolic cage partitioned into units for replication of the treatments. There were

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Table 1: Composition of the experimental diets on as fed basis (%)

Diets	1	2	3	4	5	6
Feedstuffs						
Maize	54.60	55.35	54.35	53.35	49.85	48.35
SBM	35.00	35.50	31.50	27.50	26.00	22.50
AASM	0.00	2.50	5.00	7.50	10.00	12.50
Bone meal	1.50	1.50	1.50	1.50	1.50	1.50
Oyster shell	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15
Premix*	0.50	0.50	0.50	0.50	0.50	0.50
Palm oil	6.25	2.50	5.00	7.50	10.00	12.50
Glycine	0.50	0.50	0.50	0.50	0.50	0.50
Riboflavin	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00

*Premix supplied/kg of diet, Vitamin A, 8 x 10^6 IU; Vitamin D3 1,200 IU; Vitamin E 31 IU; Vitamin k_3-kastab 2 mg, Riboflavin 3 mg, Nicotinic acid 10 mg, Panthothenic acid 150 mg, Manganese 80 mg, Zinc 50 mg, Copper 2 mg, Iodine 1.2 mg, Cobalt 0.2 mg, Selenium 0.1 mg

24-chicks per treatment replicated thrice with equal number of randomized chicks. The chicks were fed the experimental diets shown in Table 1.

At the end of the feeding trial, a bird per replicate was randomly taken and blood samples collected by decapitation into EDTA treated sample bottles to avoid clotting. Blood samples collected were analyzed for haematocrit (PCV), erythrocytes (RBC), leucocytes (WBC), Haemoglobin (Hb) and the percentage WBC differential counts of lymphocytes, eosinophils, monocytes, basophils and neutrophils (Wintrobe, 1956). The same broilers sacrificed for blood collection were bilaterally opened and the entire Gastrointestinal Tract (GIT), the caeca, jejunum, ileum and duodenum sections had their lengths taken. Organs such as the livers, lungs, pancreases, hearts and intestines were excised for the determination of absolute and relative organ weights (AOW and ROW).

Statistics: Data collected on blood composition, GIT, AOW and ROW were analyzed according to ANOVA using the one way experimental design model (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The investigation on blood indices in this study is based on the premises that ingestion of numerous dietary compounds has measurable effects on haematological constituents thereby providing a valuable means of chemically determining the presence of several metabolites and other constituents in the body of an animal. It is also a good way of assessing and diagnosing the health status of an individual or flock as it plays a vital role in the physiological, nutritional, pathological and metabolic disorders of an organism (Harper *et al*., 1979; Islam *et al*., 1999). Data recorded on haematological parameters in broilers fed dietary treated AASM presented in Table 2 shows increase in PCV and WBC as the dietary level of AASM increased indicating that treatments used for detoxification were effective in reducing or removing the toxins in AASM. Normal PCV values, which measure the number of erythrocytes per 100 ml of blood, in adult hens and cocks are reported to be 27 and 37% respectively (Bierrer *et al*., 1964), while 25-45% were reported by Miltruka and Ramselay (1977). Since variations in haematocrit is due to age, sex, breed, nutrition, climate, geographical location, season, stress, digestion, day length and other factors (Duke, 1955; Lucus and Jamroj, 1961; Calhroun and Brown, 1975), the PCV values obtained in this study appear to agree with those reported by the mentioned authors since birds used in this study were day old in age at the start of the experiment. WBC count was similarly influenced as discussed by the above factors. The normal leucocytes values in domestic mature chickens range from 24-32 or 9-32 x 10^3/mm (Lucus and Jamroj, 1961; Epelle, 1982). Leucocytes values above these normal counts is said to be due to proliferation of foreign bodies or development of abnormal physiological state in the animal (Lucus and Jamroj, 1961). In this work, the WBC values seemed to lie within the normal ranges considering the age of the broiler chicks.

Erythrocyte values on the c: Erythrocyte values gave a range of 2.17 x 10^{12}/l and an average of 1.54 x 10^{12}/l on the control diet and test diets respectively while normal RBC ranges in adult domestic fowls are given as from 2.6-3.3 x 10^9/mm (Lucus and Jamroj, 1961; Epelle, 1982). As explained earlier on PCV and WBC, taking into cognizance of the experimental birds' age and other associated factors, these values tallied with the reported normal range. Haemoglobin, the measure of amount of oxygen carrying protein in RBC, showed marginal variation between the conventional and AASM based diets. In adult male and female fowls, Dukes and Schwarte (1931) reported normal RBC concentration between 9.8 and 13.50 gm/100 ml blood and higher

Table 2: Dietary effects of chemically detoxified AASM on some haematological indices in broilers

Diets	1	2	3	4	5	6	SEM
PCV (%)	21.33^a	28.66^c	26.66^b	25.00^b	25.00^b	24.66^b	5.10
WBC ($x10^{12}$/L)	11.73^a	12.77^b	11.67^b	12.87^b	13.20^c	10.86^a	2.33
RBC ($x10^{12}$/L)	2.17^c	1.87^b	1.71^b	1.44^b	1.55^b	1.37^a	0.13
Hb (g/dl)	8.27^b	8.40^b	7.82^a	7.79^a	7.32^a	7.69^a	0.10
Lymphocytes (%)	67.00^a	64.00^a	72.00^b	67.00^a	78.00^c	70.00^b	5.26
Monocytes (%)	7.00	2.00	-	2.00	-	-	
Neutrophils (%)	33.00^c	35.00^c	28.00^b	31.00^c	20.00^a	28.00^b	3.70
Eosinophils (%)	-	3.00	-	1.00	2.00	3.00	
Basophils (%)	-	-	-	1.00	-	-	

$^{a-b-c}$Means in rows not sharing common letters differed significantly ($p<0.05$)

Table 3: Influence of detoxified AASM in diets on length of some GIT sections (cm)

Diets	1	2	3	4	5	6	SEM
Indices							
GIT	140	159	159	162	159	147^{NS}	1.94
Small intestine	96^c	90^b	91^b	88^a	97^c	108^{d*}	0.41
Caeca	18^a	21^b	22^b	19^a	18^a	17^{a*}	0.14
Jejunum	42^a	52^c	49^b	49^b	56^c	55^{c*}	0.33
Ileum	47	39	43	38	33	53^{NS}	0.67
Duodenum	8	7	8	8	9	7^{NS}	0.78

$^{a-b-c-d}$Means in rows not sharing common superscripts are significantly different ($p<0.05$). NS = Not Significant ($p>0.05$)

Table 4: Response of absolute weight of some organs in broilers fed detoxified AASM based diets relative to the control diet (g)

Diets	1	2	3	4	5	6	SEM
Organs							
Pancreases	0.87^c	0.83^c	1.00^e	0.77^b	0.93^d	0.63^*	0.18
Livers	6.67^d	4.20^b	5.10^c	4.76^b	4.47^b	3.77^{a*}	2.33
Hearts	1.20^e	0.80^c	0.96^d	0.77^d	0.87^c	0.66^{a*}	0.04
Intestines	23.33^b	21.33^b	23.67^b	19.00^a	21.00^b	18.33^{a*}	0.66
Lungs	0.67^a	0.67^a	1.33^c	9.77^a	0.63^a	0.60^{a*}	0.08

Table 5: Effects of detoxified AASM in diets on some relative organ weights in broilers

Diets	1	2	3	4	5	6	SEM
Organs							
Pancreases	0.50^b	0.06^c	0.06^c	0.04^a	0.06^c	0.04^{a*}	0.001
Livers	0.47^c	0.33^b	0.35^b	0.43^c	0.33^b	0.27^{a*}	0.002
Lungs	0.04^a	0.05^b	0.08^c	0.07^c	0.05^b	0.04^{a*}	0.01
Hearts	0.08^c	0.06^b	0.06^b	0.07^c	0.06^b	0.04^{a*}	0.01
Intestines	1.52^b	1.54^b	1.53^b	1.74^c	1.55^b	1.39^{a*}	0.23

values in male adults than in females, besides variations due to nutrition, hypoxia, time of day, hormonal and other factors, hence findings in the present work agreed with the authors. The normal Hb values on both the control diet and the test diets indicate the nutritional adequacy of the dietary processed AASM otherwise low or high values would have been obtained due to anaemia caused by nutritional deficiency, blood loss, destruction of blood cells, failure of the bone marrow to produce blood, on the other hand high Hb values could result than normal due to excessive bone marrow production of RBC or lung diseases. The five different types of WBC in the body namely lymphocytes, monocytes, neutrophils, eosinophils and basophils responsible for body defense against infections and foreign bodies were well represented among the dietary treatments especially the neutrophils and lymphocytes known to produce antibodies against harmful germs in the body. Their presence on all the dietary treatments suggests immunological security of the birds under investigation.

Statistically, significant differences were not recorded on the total gastrointestinal tracts, ileal and duodenal lengths relative to values on the reference diet (Table 3), which might suggest that the test feedstuff in diets was comparable in nutritional composition as that of the control diet. The significant differences observed on lengths of intestines, caeca and jejunum could be due to the reaction of these organ sections to the mild residual hypoglycins in AASM or other unobservable factors. Absolute and relative organ weights (AOW and ROW) of the pancreases, livers lungs, hearts and intestines of the broilers were significantly influenced by dietary AASM compared with the weight of organs on the group offered the conventional diet (Table 4 and 5).

Differences in weights of organs supported the result on performance characteristics which recorded significant increase in body weight gain of the broiler chicks fed the

Table 6: Performance characteristics showing significant differences in body weight gain among the various dietary treatments in broilers fed AASM in diets compared to the control diet

Diets	1	2	3	4	5	6	SEM
Parameters							
Avg feed intake (g/b/d)	46.52	47.33	50.30	32.57	47.88	35.38[NS]	2.67
Avg wt. gain (g/b/d)	22.48[d]	20.43[c]	23.64[c]	14.98[a]	19.29[b]	18.71[b]*	2.35
Avg daily growth rate (%)	89.95	77.69	90.54	60.00	77.10	75.00[NS]	9.06
Feed efficiency (F/G)	1.60[a]	1.80[b]	1.70[b]	2.18[c]	1.99[b]	1.92[b]*	0.02
Survival rate (%)	100.00	99.00	99.00	100.00	100.00	100.00[NS]	

different dietary treatments since these diets contained varying amount of red palm oil, some diets containing more volume than others. The differences in organ weights could also be attributable to the left over allelochemicals in ackee apple seed meal since one may not be able to attain 100% removal or reduction of anti-nutritional factors contained in a novel feedstuff.

In conclusion, results on haematological parameters, GIT, AOW and ROW of broilers following ingestion of dietary detoxified AASM suggest that the methods used offer great potentials to effectively detoxifying ackee apple toxins. Further experiments using same treatment methods but increasing the levels of AASM in diets above beyond the 12.50% used in this trial are recommended.

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