HAEMATOLOGY AND BLOOD CHEMISTRY OF LAYING HENS FED RAW *Anthonatha macrophylla* SEED MEAL BASED DIET

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ABSTRACT

Haematology and blood chemistry of laying hens fed raw *Anthonotha macrophylla* seed meal (AMSM) based diet were evaluated in an eight-week feeding trial involving one hundred and twenty (120) laying hens. Four experimental diets were formulated to incorporate AMSM at 0 %, 4 %, 8 % and 12 %, in diets A, B, C and D respectively. The laying hens were divided into four groups, each group was replicated three times in a completely randomized design (CRD). There were significant differences ($P<0.05$) in all haematological parameters measured except in red blood cell. Haemoglobin (g/dl), white blood cell ($\times 10^3$ /µL), packed cell volume and mean corpuscular volume (fl) significantly increased as the level of AMSM increased in the diet, Mean corpuscular haemoglobin (pg) and mean corpuscular haemoglobin concentration were decreasing across the treatment groups. The biochemical parameters showed significant decrease ($P<0.05$) across the groups. The obtained values in biochemical indices and haematological parameters were within the normal range acceptable for birds, indicating better health status of the experimental animals. From the obtained results, it was concluded that laying hens were able to tolerate up to 12 % level of inclusion of *Anthonotha macrophylla* seed meal without deleterious effect on their health status.

Keywords: *Anthonotha macrophylla*, Laying hens, haematology, blood chemistry

INTRODUCTION

Animal protein deficiency in the diet of Nigerians and the people of most developing countries is of great concern, and it is relevant now to take urgent measures to solve the problem of imminent protein malnutrition. Feed accounts for 70-80 % of the production cost of poultry (Opara, 1996). The bulk of the feed cost arises from protein concentrates such as groundnut cake, fishmeal and soybean meal. Prices of these conventional protein sources have soared so high in recent times that it is becoming uneconomical to use them in poultry feed (Opara, 1996, Esonu et al., 2001). There is need therefore to look for locally available and cheap sources of feed ingredients particularly those that do not attract competition between humans and livestock. It is also very important to ascertain the effect of these non-conventional feed resources on the physiological status of the animal especially the haematology and blood chemistry.

Nigeria and Africa at large are endowed with seed bearing plants, which over the years have served various purposes and yet quite a number of them remain untapped. One of such underutilized plants is *Anthonotha macrophylla* P. Beauv, a member of the family Leguminosae– Caesalpinioideae. *Anthonotha macrophylla* is a medium-sized tree up to 20m high which is common throughout the rainforest. It abounds in Nigeria with common names (Yoruba: abata; Igbo: ububa-ikpa). The various parts of the plant have several ethno-medicinal claims, the bark had been found useful in the treatment of venereal diseases and as vermifuges, the root for the treatment of intestinal related discomfort and the seeds for

general food (Ugoeze et al., 2014). Analysis revealed that this useful tree contains substantial amount of crude protein, carbohydrate, ash and fiber suggesting that the plant will definitely find place in monogastric animal feed production as a feed ingredient (Durunna, 2006). The aim of this study was to determine the effect of raw *Anthonotha macrophylla* seed meal (AMSM) based diets on the haematology and blood chemistry of laying hens.

Materials and Methods

Experimental site

The experiment was carried out at the Poultry Unit of Faculty of Agriculture Teaching and Research Farms, Abia State University, Umudike Campus. Umudike bears a coordinate of 7°31¹ East and 5°28¹ North and lies at an altitude of 122 meters above sea level.

Experimental Diets

Fresh seeds of *Anthonotha macrophylla* were harvested from the wild within Isiala Oboro in Ikwuano Local Government of Abia State. The raw seeds were sundried for 4 days, chopped and milled using a hammer mill to produce the *Anthonotha macrophylla* seed meal (AMSM).Four experimental diets were formulated by incorporating AMSM at 0, 4, 8 and 12 % dietary levels for diets A, B, C and D respectively. The composition of the experimental diets is shown in Table 1. The determined proximate composition of raw AMSM and experimental diets are presented in Tables 2 and 3 respectively.

Experimental Design

A total of one hundred and twenty (120) laying hens were used for the experiment. The birds were divided into four groups of thirty hens each and assigned to four treatment diets in a completely randomized design (CRD). Each group was further sub-divided into three replicates of ten hens each. Feed and water were offered *ad libitum* and the feeding trial lasted 8 weeks.

Blood Collection

At the end of the experiment, one bird per replicate was randomly selected making a total number of 12 birds. The sampled birds were bled using sterilized knife through the jugular vein to aspirate 7mls of blood from each bird for which 2mls were collected into bijour bottle treated with ethylene diamine tetra acetic acid (EDTA) for haematological assay (packed cell volume, haemoglobin, white blood cell and red blood cell) and 5mls of blood each were collected into EDTA free bottles for serum biochemistry (total protein, blood glucose, blood urea and serum enzymes).

Data Analysis

Data obtained were subjected to statistical analysis using one-way analysis of variance (ANOVA) as outlined in Steel and Torrie (1980). Duncan multiple range test was used to separate significant treatment means where they occurred (Obi, 2002).

Table 1: Composition of Experimental Diets (%)

	AMSM Inclusion Levels in Diets (%)			
Ingredient	0(T_1)	4(T_2)	8(T_3)	12(T_4)
Maize	50.00	50.00	50.00	50.00
Soybean meal	20.00	16.00	12.00	8.00
AMSM	0.00	4.00	8.00	12.00
Fish meal	3.00	3.00	3.00	3.00
Wheat bran	15.00	15.00	15.00	15.00

Palm kernel cake	7.00	7.00	7.00	7.00
Bone meal	3.00	3.00	3.00	3.00
Salt	0.50	0.50	0.50	0.50
Premix*	0.50	0.50	0.50	0.50
Methionine	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Calculated composition				
Crude protein (%)	19.50	18.19	17.22	17.30
Metabolizable energy (MJ/kg)	11.30	11.39	11.55	11.71

*Vitamin mineral premix provides per kg diet: Vit. A, 13.340 iu, Vit D_3 2680 iu, Vit E 10 iu, Vit. K, 2.68 iu, Calcium penthenate, 10.68mg, Vit. B_{12} 0.022mg; Folic acid, 0.668mg; Chorline Chloride 400mg; Chlorotetracycline, 26-28mg; Manganese, 133.34mg; Iron, 66.68mg;Zinc, 53.34mg Copper, 3.2mg;Iodine, 1.86mg; Cobalt, 0.268mg; Selenium, 0.108mg. AMSM = *Anthonotha macrophylla* seed meal.

Table 2: Determined proximate composition of experimental diets

	AMSM inclusion levels in the diets (%)			
Parameter	0(T_1)	4(T_2)	8(T_3)	12(T_4)
Dry matter (%)	89.74	90.17	90.26	90.09
Crude protein (%)	17.32	17.27	17.19	17.01
Crude fibre (%)	8.47	7.94	7.71	6.82
Ether extract (%)	3.17	2.97	3.31	3.21
Ash (%)	6.45	5.86	7.27	8.36
Nitrogen free extract (%)	64.59	55.46	54.78	54.69
Metabolizable energy (MJ/kg)*	13.35	11.92	11.95	11.84

AMSM – *Anthonotha macrophylla* seed meal, NFE – Nitrogen free extract, NFE – Nitrogen free Extract, ME – Metabolizable energy, *Calculated according to Pauzenga as ME (MJ/kg) = 37 × % CP + 81 × % EE + 35.5 × % NFE.

Table 3: Proximate composition of raw *Anthonotha macrophylla* seed meal (AMSM)

Parameters	Raw AMSM
Dry matter (%)	90.51

Crude protein (%)	20.88
Crude fibre (%)	3.81
Ether extract (%)	6.25
Ash (%)	8.71
Nitrogen free extracts (%)	60.35
Metabolizable energy (MJ/kg)	14.31

Results

Haematological parameters of laying hens fed different levels of raw *Anthonotha macrophylla*.

The results of the haematological indices are as presented in Table 4. The parameters recorded showed significant differences (P<0.05) among the treatment groups except in the red blood cell count. There were increases in the values of haemoglobin, white blood cell, packed cell volume and mean corpuscular volume as the level of AMSM increased in the diet. The mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration decreased with an increase in the level of AMSM in the diet. The red blood cell count was not affected by the dietary inclusion of *Anthonotha macrophylla* seed meal as feed ingredient.

The results of biochemical indices of laying hens fed *Anthonotha macrophylla* seed meal are as presented in Table 4. Biochemical parameters measured such as serum glucose, cholesterol, urea, creatinine and aspartate transaminase (AST) significantly increased (P<0.05) as the level of AMSM increased in the diet. The serum albumin showed no significant difference (P>0.05) among the treatment groups.

Table 4: Haematological parameters of laying hens fed graded levels of raw *Anthonotha macrophylla* seed meal

	AMSM inclusion levels in diets				
Parameter	0 (A)	4 (B)	8 (C)	12 (D)	SEM
Hb (g/dl)	11.00^{b}	12.50^{ab}	12.73^{ab}	13.00^{a}	0.22
RBC($\times 10^{6}$µ/l)	2.81	2.74	2.52	2.27	0.22
WBC ($\times 10^{3}$µ/l)	150.80^{b}	170.00^{a}	180.70^{a}	183.70^{a}	4.45
PCV (%)	29.67^{b}	37.17^{a}	37.00^{a}	39.00^{a}	0.35
MCV (fl)	129.33^{b}	130.40^{b}	135.33^{a}	135.20^{a}	0.24
MCH (pg)	47.83^{a}	45.23^{b}	40.70^{c}	39.03^{c}	0.49
MCHC (%)	36.67^{a}	36.07^{a}	34.03^{b}	32.33^{c}	0.01

$^{a\,b\,c\,d}$ Means in the same row with different superscripts differed significantly (p<0.05).

AMSM - *Anthonotha macrophylla* seed meal.

SEM-Standard error of the means, RBC= Red blood cell, WBC= White blood cell, PCV= Pack cell volume, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration.

Table 5: Biochemical Parameters of laying hens fed diets containing graded levels of AM

	AMSM Inclusion Levels in Diets (%)

	T_1(0)	T_2 (4)	T_3 (8)	T4 (12)	**SEM**
Glucose (mg/dl)	207.01[d]	230.00[c]	250.82[b]	268.90[a]	0.57
Cholesterol (mg/dl)	190.01[d]	195.22[c]	198.01[b]	201.00[a]	0.58
Total protein (mg/dl)	7.87[a]	6.50[b]	6.10[c]	5.37[d]	0.01
Albumin (mg/dl)	2.90	3.01	3.00	3.80	0.02
Globulin (mg/dl)	4.63[a]	3.44[b]	3.08[c]	2.10[d]	0.31
Urea (mg/dl)	43.50[c]	45.10[c]	59.22[b]	70.20[a]	0.60
Creatinine (mg/dl)	1.30[b]	1.35[b]	2.00[a]	2.03[a]	0.23
AST (iu/L)	190.00[d]	195.85[c]	200.75[b]	208.33[a]	0.48

[a b c d] Means in the same row with different superscripts differed significantly ($p<0.05$).

AMSM = *Anthonotha macrophylla* seed meal, AST = Aspartate transaminase.

SEM-Standard error of the means

Discussion

Haematological Parameters of Laying Hens Fed AMSM

Haematological and biochemical parameters are important indicators of health status in animals and have been an indispensable tool in the diagnosis, treatment and prognosis of many diseases (Emiola et al., 2013). The haemoglobin content of laying hens fed AMSM, ranged from 11.00g/dl in diet A to 13.00g/dl in diet D. Haemogloblin values reported in this study fall within the range of 11.81-13.32mg/dl reported for laying Japanese quails by (Ogbuewu, *et al.* 2014). The values were also comparable with the range of 11-14.00g/dl reported for broiler turkey by (Ugwuene, 2011).

The red blood cell count was not affected by the inclusion of AMSM in the diet. The values obtained were within the normal of 2.5-3.5 ($\times 10^6$ /µL) reported for birds (Jain, 1993). Red blood cell is known to transport oxygen in the body.

The white blood cell values obtained in this study were above the normal range of 20-30 ($\times 10^3$/ µL) reported for birds (Radostits et al., 1997). The increase in the white blood cell could be attributed to the presence of foreign materials such as anti-nutrients in the diet which the cells were combating, since the WBCs are known to fight against foreign substances in the body (Murray et al., 1993; Harper et al., 1999).

The packed cell volume values (29.67-39.00%) in this study were within the normal range of 22-35% reported for birds (Jain, 1993), and also within the normal range of 26.00 – 45.20% for normal adult chicken (Mitruka and Rawnsley, 1977). This thus implies that the feed value of the diets was adequate and the inclusion of *Anthonotha macrophylla* in the experimental diets did not reduce its nutritional quality. The mean corpuscular volume (MCV) values reported in this study were within the range of 90-140fl reported for birds, which implied that birds may neither stand the risk of haemaconcentration nor anaemia (Frandson, 1981).

Mean corpuscular haemoglobin values of laying hens ranged from 39.03-47.83pg, these were below the range of (53.10-97.00pg) reported for turkey by (Ugwuene, 2011) but higher than 25-27pg reported for chicken (Reece and Swenson, 2004).The MCH value obtained in birds fed the control diet (47.83pg) was significantly greater ($P<0.05$) than other groups followed by that of diet B (45.23pg), while that of

diet D (39.03pg) was the least. Since mean corpuscular haemoglobin is an indicator of the oxygen carrying ability of the red blood cell (Frandson, 1981), the blood of laying hens fed the control diet A and diet B may perform respiratory functions more efficiently than the other groups. The mean corpuscular haemoglobin concentration values obtained in this study decreased as the level of AMSM increased in the diet. The values obtained compared with the normal range of 26-35g/dl reported for birds (Jain, 1993). High levels of MCHC can lead to lack of B_{12} or folic acid in the body of the animal. The observed increase of haematocrit in this study contradicts the findings of (Ogbuewu et al., 2014) who observed decline of haematocrit of Japanese laying quail.

Biochemical indices of laying hens fed raw AMSM

The biochemical indices of laying hens fed varying levels of raw AMSM are as presented in Table 5. The serum glucose values (207.01-268.90mg/dl) were above the range of (173.22-206.95mg/dl) reported by Raji et al. (2000). The high glucose level obtained in this work might have resulted from the fact that birds generally appear to maintain a high and relative constant blood glucose values (Raji et al., 2000). The cholesterol range of 190-201mg/dl in this study agreed with the range of 91.95-466.11mg/dl reported by Ali et al. (2012). The increase in serum cholesterol observed in the study confirmed the findings of (Hassan, 2010) who observed an increase in serum cholesterol level with advancement in laying. The increase observed could be attributed to a general increase in lipid mobilization needed in the synthesis of liver lipids.

The serum total protein range (5.37-7.87mg/dl) compared favorably with the range of 5-7mg/dl reported for birds (Banerjee, 2005). The serum total protein decreased as the level of AMSM increased in the diet. Low serum total protein is an implication of inadequacy of dietary protein and also poor protein utilization (Egumm, 1990). Since the values were within the normal range for birds, it would seem that the dietary protein was adequate and utilized by the laying hens.

Serum albumin values ranged between 2.90-3.80mg/dl. The obtained values compared with the normal range of 2-3.5mg/dl reported for birds (Banerjee, 2005). The serum albumin of birds fed diet D (3.80mg/dl) was slightly higher than the normal range for birds, since albumin is involved in blood clotting (Frandson, 1981), birds on diet D may likely have less risk of hemorrhage than the other groups.

Serum globulin range (2.10-4.63mg/dl) obtained in this study were within the normal range of 2.1-3.7mg/dl (Banerjee, 2005), except in birds fed diet A (4.63mg/dl), whose value was higher than the normal range. High serum globulin indicates better ability to fight against diseases. Birds fed diet A would resist disease more than the other groups.

Serum urea is assumed to indicate protein breakdown and higher urea value indicates poor dietary protein utilization (Ogbuewu et al., 2014). The values (43.50-70.20 mg/dl) reported in this study were at variance with the range of 0.4-1mg/dl reported for birds (Banerjee, 2005). High blood urea is a clear indication of poor quality protein.

Creatinine values were between 1.30-2.03mg/dl. Creatinine is a product of creatinine phosphate breakdown in the muscle which is usually produced at fairly constant rate by the body depending on muscle mass (Ogbuewu et al., 2014). The serum creatinine values are comparable with the reference value (1-2mg/dl) for birds (Reece and Swenson, 2004; Banerjee 2005) and this ruled out the possibility of excessive breakdown of muscle tissue, hence the laying hens were not serving at the expense of body reserve.

The concentration of different enzymes related to metabolism and function of the heart, liver and

kidney are used as indication of their effects on these vital organs. The aspartate transaminase (AST) values were between 190-208.33 iu/L, and comparable with the range (190-209.00 iu/L) reported by Ogbuewu et al. (2014) and 88-208µ/L reported for healthy birds (Banerjee, 2005). This is an indication of normal functioning of the liver of the experimental animals. High AST is as a result of disease and morbid conditions involving injuries to large numbers of metabolically active cells.

Conclusion

The obtained haematological and serum biochemical parameters were within the recommended range for healthy birds. It could therefore be concluded that laying hens were able to tolerate up to 12 % level of inclusion of raw *Anthonotha macrophylla* seed meal without deleterious effect on their health status.

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