HEAMATOLOGY AND SERUM BIOCHEMISTRY OF LAYING HENS FED GRADED LEVEL OF PALM BUNCH ASH AS A MINERAL SOURCE

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ABSTRACT
In producing plant ash, the organic components of the material which ordinarily interact with the mineral molecule to reduce their bioavailability are completely eliminated. The end product may therefore successfully replace intact plant and rock mineral sources in animal and human diets when properly processed. Ash derived from the combustion of various parts of the palm tree such as trunk, inflorescence, bunch and leaves have been analyzed and shown to contain excellent levels of essential minerals, especially potassium, which also makes the ash highly alkaline. The objective of this study was therefore to evaluate the hematology and serum biochemistry of laying hens fed graded levels of palm bunch ash as mineral source. Empty palm bunches were used for the study. It was cleaned, sundried and burnt to produce palm bunch ash (PBA). It was analyzed for mineral concentration using atomic absorption spectrophotometer. Two hundred and forty Isa brown layer birds that were 16 weeks into lay were used to assess the hematological and serum biochemical indices when fed with graded levels of PBA that lasted for 12 weeks. They were divided into four groups of 60 birds each and further replicated three times in a completely randomized design (CRD) with 20 birds per replicate. Four experimental layer diets were formulated such that the control diets had no PBA while the other three diets had graded levels of PBA supplementation in the diets at 0. 10, 0.15 and 0.2kg/100kg of feed respectively in replacement of common salt. Proximate and mineral analysis of the feeds were determined. Blood samples were collected to determine hematological and serum biochemical characteristics. The 0.1kg/100kg diet (T2) supplemented group recorded significantly higher Hb, and PCV values than the control, and the other supplementation groups. Lipid profile results obtained in the study showed that triglyceride, especially the high-density lipoprotein (HDL), decreased in values as the PBA supplementation level increased except at T4. These positive results were supported by superior hematological and serum biochemical indices of T2 and T3 birds.
Keywords: Laying hens, Palm bunch ash, Minerals, Poultry feed, blood characteristics

INTRODUCTION
Mineral electrolytes are commonly known to support a host of body physiologic functions and processes including synthesis of tissue proteins, maintenance of intracellular and extra cellular homeostasis, maintenance of ionic potential across cell membranes and organelles, driving of enzymatic reactions, osmotic pressure regulations and acid base balance among others (Borges *et al*., 2004; Unamba- Oparah *et al*., 2017). They are therefore essential for optimizing animal nutrition, health, physiology and biochemistry. Among these electrolytes, the monovalent ions (K+, Na+, Cl-) are the key ones involved in acid- base balance of the fluids, because they have higher permeability and greater absorption potential in the digestive tract than divalent ions (Unamba- Oparah *et al*., 2017). Recently ash supplementation has been shown to enhance dietary mineral elements absorption and edible carcass development in pullets (Okoli et al., 2014; Nwogu *et al*., 2021). Ohanaka *et al*., (2022) reported better growth efficiency ratio (GER) in broilers at all growth phases of dietary palm kernel shell ash supplementation, indicating enhanced nutrient utilization. This study therefore evaluates the effects of palm bunch ash supplementation on the hematology and serum biochemistry of laying hens.

MATERIALS AND METHODS
Empty palm bunches were collected from an oil palm mill at Umuagwo, Ohaji Egbema local government area of Imo state. They were sundried, burnt and the ash sieved and particles removed. Mineral composition of the palm bunch ash was done at Precision laboratory, Ibadan, Oyo state. Four

experimental layer diets were formulated such that the control diets had no PBA while the other three diets had graded levels of PBA supplementation in the diets at 0. 10, 0.15 and 0.2kg/100kg of feed respectively in replacement of common salt. All other ingredients were of equal proportion across the test diets. Two hundred and forty (240) Isa brown layer birds that are 16 weeks in lay were used for the experiment that lasted for 12 weeks. Blood was collected and emptied into sterilized bottles containing Ethylene diamine tetra acetic acid (EDTA) for hematological analysis. Another set was collected in plain tubes without EDTA for serum biochemistry. Hematological parameters such as packed cell volume, red blood cell, white blood cell and hemoglobin were determined. Serum biochemical parameters such as total serum protein, albumin and globulin), urea, creatinine and cholesterol were determined. All data collected were analyzed statistically using Analysis of variance (ANOVA) and differences between the treatment means were compared using the Duncan's Multiple Range Test using Statistical package for social Sciences (SPSS) user's guide, Version 24.00 (SPSS,2012).

Table1: Ingredient composition of PBA supplemented diets in layer birds (100kg)

Ingredients	T1 Control	T2 0.10% PBA	T3 0.15% PBA	T4 0.20% PBA
Maize	44.00	44.00	44.00	44.00
Wheat offal	19.00	19.00	19.00	19.00
Soya bean meal	16.00	16.00	16.00	16.00
Brewers Dried Grain	5.00	5.00	5.00	5.00
Palm kerenl cake	4.00	4.00	4.00	4.00
Fish meal	1.60	1.60	1.60	1.60
Limestone	7.00	7.00	7.00	7.00
Bone meal	2.50	2.50	2.50	2.50
Premix	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20
Common salt	0.25	0.15	0.10	0.05
Ash	-	0.10	0.15	0.20
Total	100	100	100	100
Nutrient calculated				
Crude protein	16.20	16.20	16.20	16.20
Crude fibre	5.7	5.7	5.7	5.7
Met. Energy (ME. Kal/kg)	2650	2650	2650	2650

Vitamin premix contains the following per kg of feed: Vit A = 5,000,000IU, Vit D3 = 1,000,000IU, Vit E = 1875IU, Vit K = 1255gm, Thiamin (B1) = 0.6255gm, Riboflavour = 1.875gm, Calcium panthothenate = 2.8kg, Nicotinic acid = 5.625gm, Pyridoxin = 0.625gm, Vit B12 = 5gm, folic acid = 0.31gm, Biotin = 0.1gm, Cholin chloride = 150gm, methionine = 75gm. Manganese = 5gm, Iron = 10gm, Copper = 1.5gm, Iodine = 0.5gm, Cobalt = 1.0gm, Selenium = 0.05gm, Antioxidiane 50gm, Antimold = 7.5gm, Nigrovin = 10gm, lysine = 75gm.

RESULTS AND DISCUSSION

The results of the effects of different supplementation levels of PBA on the hematological indices of the laying hens are presented in table 2. The results showed that the PBA supplementation resulted in significant ($p<0.05$) increases in the values of Hb, and PCV up to T3, before decreasing significantly below ($p<0.05$) the control value. Thus, the 0.1kg/100kg diet (T2) supplemented group recorded significantly higher Hb, and PCV values than the control, and the other supplementation groups. The T3 (0.15kg/100kg) group also recorded significantly higher values than the T4 (0.2kg/100kg) group. T4 therefore recorded the lowest values for the two parameters. The Hb values (9.10 -10.70g/dl) obtained in this study are in agreement with the range of values (8.7 – 11.3g/dl) reported by Nwogu (2013) in pullets fed plantain ash supplemented diets. The PCV values fell within the normal reference range values (30 – 40%) published by Aiello and Mays (1998). Interestingly, there were no significant treatment effects on the RBC values although there was again a progressive numerical

increase in values up to T3 (2.32x 10^6/L), before the drop to 2.07x 10^6/L. Invariably, all the SFA supplemented groups recorded numerically superior values to the control group. The RBC values obtained in this study are however lower than the reference values published by Aiello and Mays (1998). The control values of the MCV decreased significantly as a result of SFA supplementation ($p<0.05$), while for the MCH, and MCHC, the control values were significantly higher ($p<0.05$) than those of T2 and T4 but similar to T3 ($p<0.05$). Therefore, the T4 group recorded significantly lower values across all hematological parameters measured.

Table 2: Hematological characteristics of laying hens fed PBA supplemented diets

Parameters	T1 Control	T2 0.10% PBA	T3 0.15% PBA	T4 0.20% PBA	SEM
Hb (g/dL)	9.20^{c}	10.70^{a}	9.90^{b}	9.10^{c}	0.20
PCV (%)	26.80^{c}	30.20^{a}	27.73^{b}	25.80^{d}	0.49
RBC $X10^{12}$/L	1.95	2.31	2.32	2.07	0.07
MCV (fl)	137.40^{a}	130.70^{c}	133.70^{b}	128.40^{d}	1.02
MCH (pg)	47.27^{a}	46.30^{b}	47.50^{a}	45.30^{d}	0.27
MCHC (g/dL)	47.20^{b}	46.30^{c}	47.60^{a}	45.30^{c}	0.27

Means with different superscript on the same horizontal row are significantly different @P< 0.05

Table 3 shows the results of the serum biochemical analysis. The PBA treatments resulted in significant ($p< 0.05$) changes in the serum concentrations of total serum protein (TSP), albumin, and globulin from the control values. The concentrations of TSP, and globulin were highest in T2, and thereafter decreased significantly in T3 and T4. Although the reverse trend was observed for the albumin, the T3 value was unexpectedly higher than the T2 values. The TSP values in this study were higher than the ranges of 3.10 -3.70 g/dl reported by Nworgu *et al*. (2007), when fluted pumpkin leaf extract was added to broiler ration, but much lower than the range of 7.25 – 10.15g/dL reported by Okoli, et al., 2014 in plantain ash fed hens. The decreasing value of the TSP in this study is probably reflective of the higher percentage hen day production in the ash supplemented groups, which invariably resulted to the decline in their serum values. The globulin values obtained in this study (2.20 -3.40g/dl) were within the normal reported range of 1.8 – 3.8g/dL for the chicken (Aiello and Mays, 1998). The albumin/globulin ratio expectedly followed a similar trend, with the serum albumin concentration indicating its influence on laying performance. Serum urea concentrations decreased progressively with the increasing ash supplementation in the diets. Thus, control diet value was significantly higher ($p<0.05$) than the ash supplemented diets values. The serum glucose concentration differed across the groups with PBA treated birds having significantly higher values than the control ($p< 0.05$). Increase in serum glucose concentration has been associated with increase in percentage hen- day production (Ekunseitan *et al*., 2017), which is also observed in the present study. Lipid profile results obtained in the study showed that triglyceride, especially the high-density

Table 3: Serum biochemical indices of laying hens fed PBA.

Parameters	T1 Control	T2 0.10% PBA	T3 0.15% PBA	T4 0.20% PBA	SEM
TSP (g/dl)	4.80^{b}	5.30^{a}	4.60^{c}	4.20^{d}	0.12
Albumin (g/dL)	2.00^{b}	1.90^{c}	2.30^{a}	1.80^{c}	0.06
Globulin (g/dL)	2.80^{b}	3.40^{a}	2.20^{c}	2.40^{c}	0.14
Albumin/ Globulin ratio	0.71^{b}	0.57^{b}	1.06^{a}	0.75^{b}	0.06
Urea (mg/dlL)	13.00^{a}	6.00^{b}	2.00^{c}	3.00^{c}	1.31
Glucose (mg/dL)	93.00^{ab}	101.00^{a}	96.00^{a}	98.00^{ab}	0.73
Total cholesterol(mg/dL)	158.00^{b}	151.00^{c}	191.00^{a}	137.00^{d}	5.98
Triglyceride (mg/dL)	583.00^{a}	521.00^{b}	521.00^{b}	508.00^{c}	8.94
HDL (mg/dL)	3.00^{b}	2.00^{c}	2.00^{c}	4.00^{a}	0.26
LDL (mg/dL)	38.00^{c}	45.00^{b}	85.00^{a}	32.00^{d}	6.26
Creatinine (mg/dlL	0.90^{a}	0.50^{c}	0.50^{c}	0.70^{b}	0.06

Means with different superscript on the same horizontal row are significantly different @P< 0.05

lipoprotein (HDL) decreased in values as the PBA supplementation level increased except at T4.It could be inferred that 0.2kg/100kg dietary supplementation resulted in significant increases in HDL, and decrease in LDL below control levels in the T4 hens. The serum cholesterol result followed a similar trend. A similar lowering of cholesterol levels with increasing plantain ash supplementation was reported by Nwogu et al., 2018 in broiler chickens. There was a sharp drop in the values of serum creatinine levels in response to the PBA supplementation. This is in agreement with the report of Nwogu et al., 2014 in plantain ash supplemented layers, although the range of values (0.70 – 1.80mg/dl) were much higher than the present range (0.50 – 0.90mg/dl).

CONCLUSION

Palm bunch ash supplementation did not have any adverse effects on the hematological and serum biochemical characteristics. It, however elicited superior Hb, PCV and serum glucose concentration over the control bird values.

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