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Haematology and Serum Metabolites of Pre-laying Isa Brown Hens fed Varying Dietary Levels of Alligator Pepper (Aframomum melegueta, k. Schum) Seed Meal

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Abstract
One hundred and ninety-eight (198) day old Isa Brown pullets were raised on six (6) Alligator pepper (*Aframomum melegueta*) seed meal (APSM)-based diets till 16th week when 108 hens at point of lay (POL) were fed layers mash till 18th week. The study assessed the haematological indices and serum metabolites of pre-laying Isa Brown hens fed diets with APSM. In a completely randomized design, 108 hens at POL were allotted to six experimental treatments or diets designated as T_1, T_2, T_3, T_4, T_5 and T_6. T_1 served as the control while T_2, T_3, T_4 T_5 and T_6 contained 0.1, 0.2, 0.3, 0.4 and 0.5% APSM respectively. Each treatment was replicated three times with six birds per replicate. 2.0 ml blood samples were collected from wing veins of the hens for haematological and serum metabolite analyses. There were no significant differences ($p<0.05$) in the mean values of Packed Cell Volume (PCV), white blood cells and platelets. Significant differences ($P<0.05$) in mean values were obtained for Haemoglobin, red blood cell counts, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC), but the variations were not consistent with the level of inclusion across the dietary treatments. Significant differences ($p<0.05$) also existed in the values of serum total protein, albumin and globulin, although the levels of significance did not follow the rate of inclusion levels. However, all the mean values obtained in this study were within the normal reference values reported in the literature for healthy birds except for some liver enzymes, ALT, AST and ALP which increased slightly due to stimulatory effect of the APSM on digestive system. The study concluded that 0.2% (T_3) of APSM inclusion was the best in terms of normal haematological and serum biochemistry profile and therefore, recommended further research with Alligator pepper on the laying process of other avian species.
Keywords: Haematology, Serum metabolites, Alligator pepper, Isa Brown, Pre-Laying hens
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1. Introduction

Poultry production presents the fastest means of correcting the shortage of animal protein in Nigeria due to its high carcass yield, short generation period, efficient feed utilization, small space requirement and ease of management (Owen *et al.,* 2013).

The production of poultry products such as meat and eggs has been sustained with the use of antibiotics, hormones and enzymes as growth promoters, usually at the therapeutic doses in feeds and water in order to improve the quality of the products (Plail, 2006).

Although birds raised with these synthetic performance enhancers gave good results but their potential side effects present a public health challenge worldwide, thus leading to their ban by the European Union in 2006 (Donoghue, 2003). As a replacement, extensive and considerable research into plant-based substances has been experimented with herbs and spices such as garlic, ginger, turmeric, onions, cinnamon, bitter leaf and scent leaf to mention a few.

According to Ali *et al* (2008), these herbs are commonly used home spices with unique smell and taste for several cuisines and are noted for confirmed possession of compounds with varying biological activities that are responsible for antioxidant, antimicrobial, anti-inflammatory, immuno-modulatory, pharmacological and medicinal properties. (Sofowora, 1993). Besides, these herbs have been added to the arrays of non-antibiotic growth promoters in line with the established ones such as organic acids, probiotics and prebiotics (Windisch, 2007).

Jahan *et al* (2008) reported that herbs have been used as additives in animal feeds due to their suitability and preference, lower cost of production, reduced risk of toxicity, minimal health hazards, environmental friendliness as well as their contribution to proven weight gain, feed efficiency and lower mortality in poultry.

Though reasonable levels of research have been conducted with the earlier mentioned herbs and spices in animal nutrition, specifically poultry, not quite appreciable studies are done with Alligator pepper (*Aframomum*

melegueta) and other available indigenous herbs.

Alligator pepper, with different local coinages as Grains of Paradise or Guinea Grains, is an aromatic plant. It is native to the tropical West African region and has botanical classification within the family of Zingiberaceae (Afolabi and Eko, 2016).

Literature have reported that Alligator pepper is loaded with several phytochemical substances that enhance its status as spice, as food, medicine and used in many traditional and social functions as wedding, meetings, burials, as well as aphrodisiac agent (Kamtchouing *et al.*, 2002). Its possession of both antimicrobial and anti-inflamatory properties allows for its local application in several ailments (Doherty *et al*., 2010). Alligator pepper is also used in the preparation of native pepper soup, as flavouring agent in beer, wine and gin production (Echo *et al*., 2012; Afolabi and Eko, 2016).

In addition, Alligator pepper possess useful medicinal and other therapeutic properties which are attributed to some phytochemicals that are responsible for its patho-physiological activities in the body, especially in the treatment of pains, inflammation, infectious diseases, elimination of free radicals and gastrointestinal disorders (Inegbenebor *et al*., 2009; Famuyide *et al*, 2011). Phytochemicals are non-nutritive plant substances that have disease preventive and protective properties (Alaje *et al*., 2014).

Alligator pepper seeds have been reported to contain 85.47% dry matter, 7.18% crude protein, 2.55% ether extract, 2.49% ash, 5.50% crude fibre, 73.63% total carbohydrate as well as being a rich source of minerals (mg/kg): Calcium, Phosphorus, Magnesium, Iron, Zinc, Manganese, Potassium, Sodium and Copper (Alaje *et al*, 2014). Alligator pepper seeds have also been found to contain several phytochemicals as phenol and flavonoids (Okwu, 2005), terpenoids, alkaloids, tannins and saponins (Alaje *et al*., 2014).

Given the need to research on phytochemicals with lower cost and efficacious biological values that can enhance affective poultry production, the present study was designed to investigate the effects of Alligator Pepper Seed Meal (APSM) on haematological indices and serum metabolites of Isa Brown laying birds.

2. Materials and Methods

2.1 Experimental Site

The study was carried out in deep litter pens within the Poultry Unit of the Department of Animal Science, University of Uyo Annex Campus, Akwa Ibom State, Nigeria. Uyo is located between latitudes 4^0 58'N and 5^0 04'N and longitudes 7^0 51'E and 8^0 01'E in the humid coaster rainforest of southern Nigeria.

2.2 Experimental Birds, Diets and Management

One hundred and ninety-eight (198) day old Isa Brown pullets were randomly divided into six treatments designated as T_1, T_2, T_3, T_4, T_5 and T_6 were raised with chick mash that contained 18% protein and 2816 – 2821 kcalME/kg (Afolabi and Eko, 2016) and grower mash till they reached Point-Of-Lay (POL) at 16-weeks of age in a completely randomized experimental design. One hundred and eight (108) POL hens were selected for this study and assigned to six treatments designated as T_1, T_2, T_3, T_4, T_5 and T_6 as continuation of the dietary levels of APSM from the starter and grower phases. Each treatment was replicated three times with six hens at POL in deep litter pens within the Poultry Unit of the Department of Animal Science, University of Uyo Annex Campus, Akwa Ibom State, Nigeria.

Fresh Alligator pepper seeds purchased from local market in Ibadan, Oyo State, Nigeria were removed from their epicarp, sun-dried and then milled into fine powder known as Alligator Pepper Seed Meal (APSM). The APSM used in this study contained 91.8% dry matter, 9.7% crude protein, 6.5% ether extract, 6.8% ash, 29.6% crude fibre, and minerals (mg/100g) included Calcium (240.0), potassium (35.0), phosphate (170.0), Iorn (11.2), copper (0.8) and ascorbic acid (11.0). Other constituents were 333.0µg/100gβ-carotene, 440mg/100g alkaloid and 40.0mg/100g flavonoids (Afolabi and Eko, 2016). These compounds were analyzed using AOAC methods (2000). The Six (6) experimental diets designated as T_1, T_2, T_3, T_4, T_5 and T_6 contained 0, 0.1, 0.2, 0.3, 0.4 and 0.5% of APSM as shown in Table 1. The experimental design is a completely randomized experimental design.

2.3 Experimental Birds' management

Pullets within each replicate were fed formulated and compounded feeds, provided with sufficient water and vaccinated against deadly diseases (New castle, Infectious bursal, coccidiosis and fowl pox diseases) from day old till maturity in already cleaned, fumigated, and partitioned pens.

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2.4 Data Collection

At the end of the experiment 18th week), blood samples were collected from the wing veins for haematology and serum metabolite analysis. Blood samples for the haematological procedures were collected into sterile tubes containing EDTA (an anti-coagulant) while that of serum metabolites contained no EDTA so as to enhance blood clotting, and serum so obtained was decanted for analysis.

Parameters measured were: Pack Cell Volume (PCV) counts and Red Blood Cells Counts which were

determined as described by Mitruka and Rawnsley (1977) and Ewuola and Egbunike (2008). Total White Blood Cell counts were determined with Neubauer haemocytomete upon appropriate dilution. Haemoglobin was determined with Haemoglobin scale while its constants: Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were determined with appropriate formulae (Jain, 1986).

Serum Total Proteins was analyzed by Biuret Method (Reinhold, 1953). Albumin was determined by Bromocreosol Green (BCG) method as described by Doumas and Biggs (1971). Globulin values were obtained by subtracting Albumin from the Total Protein Values (Mitruka and Rawnsley (1977) while the Albumin/Globulin ratio was obtained by dividing the Albumin value by the calculated Globulin value. Liver enzymological indices (AST, ALT and ALP) were determined with the commercial kits by colorimetric method as described by Duncan *et al*, (1994).

2.5 Data Analysis

Data generated were subjected to statistical analysis using SPSS software (SPSS, 2001) and means were separated ($p<0.05$) by Duncan Multiple Range Test (Duncan, 1995). The test ingredients and their formulations are presented in table 1 of this study below.

3.0 Results and Discussion

3.1 Haematological indices of Isa Brown layers fed graded dietary levels of APSM

Results on the haematological indices of Isa Brown layers fed graded dietary levels of APSM (Table 2) showed no significant effect ($P>0.05$) on PCV across treatments as the values obtained were within normal ranges for normal healthy hens; and may suggest the adequacy of the nutrition to the laying birds. PCV is an index of toxicity reduction in the blood and usually suggest presence of a toxic factor with adverse effect on blood formation. As there was no adverse effect on the PCV, this study was in line with the report of Ala Al Deen (2007) who observed that spices in broilers showed no significant difference on their PCV.

There was significant difference ($P<0.05$) in values of Haemoglobin (Hb) and Red Blood Cell (RBC) counts in T_3 (0.20%) compared with other treatments. Values for these parameters were within normal ranges of 7.0 – 13.0 (g/dl) for Hb and 1.58 – 3.83 for RBC (Jain, 1993; Mitruka and Rawnsley, 1997). The RBC counts of 3.13 and 3.10 observed in T_4 and T_6 of this study were significant from other treatments, implying that APSM may be haematinic to broilers. Hb and RBC count revealed that the laying birds showed no anaemic condition nor depressed respiratory capability as normal haemoglobin activity of transporting oxygen was not affected. However, the result from this study differed from the report of Ala Al Deen (2007) who reported that garlic spice fed to broilers showed significant difference ($p<0.05$) in RBC counts.

No significant difference ($P>0.05$) in the values of white blood cell (WBC) counts was observed across the dietary treatments. Although hens on T_4(0.3% APSM) and T_6(0.5% APSM) recorded the lowest values of 13.33 and 12.67 respectively, and the values were within the normal range of 9.20-28.6 ($x10^3/mm^3$). This observation might be indicative of the potentials of the APSM to have positive effect on leukopoiesis vis-à-vis a well-adapted immune system to fight infections. Confirmation to this effect was the low mortality incident recorded in this study. Animals with low WBC counts are exposed to risk of infections while those with high WBC counts are capable of generating antibodies by the process of phagocytosis, thus possessing high degree of disease resistance (Soetan *et al*. 2013).

Results on WBC counts in this study agreed with the report of Onu (2013) who observed no significant difference on similar parameter when herbal spices were fed to broilers.

Also, the platelet counts showed no consistent trend and significant difference ($P>0.05$) across the dietary treatments. Values for platelet counts were within the reference values of 9-60.4 documented by Mitruka and Rawnsley (1977) for healthy chickens, thus suggesting that cases of Thrombocytosis and Thrombocythemia associated with increase in platelet counts were prevented; while hemorrhage associated with decrease in platelet counts was not encountered.

The best results for MCV, MCH and MCHC were obtained in T_2(0.10%), T_3(0.20%) and T_5(0.40%). Significant numerical values were observed in these treatments as compared to other inclusion levels. The values obtained in this study were within the normal reference values of 102-135μ^3 for MCV, 31.9-50uug for MCH and 25.9-40.9% for MCHC reported by Mitruka and Rawnsley (1977) for chicken.

The importance of MCV, MCH and MCHC lies in their use in the diagnosis of anaemia and as an index of capacity of bone marrow to produce RBCs (Aletor and Egberongbe, 1992). The results of this study also agreed with the report of Ala Al Deen (2007) who observed that spices fed to birds showed no significant reduction in MCV, MCH and MCHC beyond the normal range, implying that APSM was not haematoxic but rather haematinic in reference to haemoglobin activity.

3.2 Serum metabolites of laying Isa Brown birds fed varying dietary levels of APSM

The serum metabolites of laying Isa Brown birds fed graded dietary levels of *Aframomum melegueta* seed meal is as presented in Table 3 below. Parameters studied were total protein, albumin, Globulin, Alanine Aminotransferase (ALT), Aspartate Aminotranferase (AST) and Alkaline Phosphatase (ALP).

Significant differences ($p<0.05$) were observed in total protein, albumin and globulin. Although there were no consistent trends in the values across the dietary treatments; the values obtained in this study were within normal reference ranges for avian species as reported by Jain (1993). For total protein, and albumin the significant difference ($p<0.05$) was recorded in T_1(0.00%) T_2 (0.10%) and T_5 (0.40%) compared to other experimental treatments. The implication of this was that the laying birds with low values in total proteins, and albumin might be affected by liver disorder while those with higher values could utilize and synthesize their proteins due to the stimulatory effects of APSM on proteolytic enzymatic system. The result of this study is at variance with the report of Al-Harthi (2006) who observed that spices fed to broiler birds had no significant effect on total proteins and that of Tijjani and Luka (2013) who reported that *Aframomum melegueta* fed to rats showed no significant change in albumin.

For globulin, values obtained across dietary treatments were significant, especially in T_5(0.40% APSM) while the control (0.00% APSM) recorded the least in value. All the values obtained for globulin were within normal range of 0.35-4g/dl as reported by Mitruka and Rawsley (1977). Increased serum globulin concentration observed in this study might be attributed to the immunostimulant effect of the APSM as noted by Al-Homidan (2005). Increased levels of globulin are also known to be associated with immune mediated diseases (Kristina, 2015).

ALT, AST and ALP values were significantly different ($p<0.05$) across the treatments, with T_1(0.00%) showing the lowest values. Values for these parameters fell within the normal ranges. Lowered ALT is an indication of non-toxic effect of the APSM on the liver while increased levels show liver cell inflammation and degeneration (Habibi *et al*. 2014). The present study does not agree with the report of Mansour *et al* (2002) who earlier observed no significant effect on serum ALT in rats fed *Aframomum melegueta.*

Also, low AST observed was suggestive of APSM non-hepatotoxic effect on the liver and kidney and this is an index of protection of these organs against hepatocellular degeneration (Al-Jaff, 2011) while higher levels of inclusions could result in hepatotoxicity (Ewuola and Egbunike, 2008). Nonetheless, this study is in agreement with the report of Ghang and Ismail (2013) who observed increase in the serum AST levels in broiler birds fed spice essential oils.

Ewuola and Egbunike (2008) aptly observed in their separate studies that increase in ALT and AST are clinical indications of diagnosis state of damage done to visceral organs, liver and kidney by toxic substances or infections.

ALP also showed decrease in the control group (0.00% APSM) compared with other experimental treatment. Even though the trend was not consistent, the values were within the normal range of 90-400µ; indicating the normal flow of bile to the liver. Higher levels of APSM could result in toxicity. However, the present study disagreed with the report of Mansour *et al* (2002) who observed that *Aframomum melegueta* had no non-toxic effects on serum ALP when herbal spices were fed to rats.

Normal ranges for both haematological indices and serum metabolites could be influenced by age, sex and physiological status of birds, since several reports indicated that blood parameter could increase or decrease with age, breed and sex (Ibrahim *et al*. 2011).

4. Conclusion and recommendation

The results from this study revealed that 0.20% inclusion of APSM in diet is safe, beneficial as well as promoting healthy haematological and serum metabolites with the attendant effects of improving immunity, performance and reproductive ability in laying birds.

Higher levels, on the other hand, could result to detrimental effects such as anaemia and hepatotoxicity. Further trials with the laying process of other avian species are suggested.

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Table 1: **Percentage composition of experimental diet with Alligator pepper fed to Isa Brown layers**.

Parameters	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**
Maize	52.37	52.37	52.37	52.37	52.37	52.37
Soybean meal	21.63	21.63	21.63	21.63	21.63	21.63
Wheat offal	3.15	3.15	2.95	2.85	2.75	2.65
Palm kernel cake	12.00	12.00	12.00	12.00	12.00	12.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Oyster shell	7.00	7.00	7.00	7.00	7.00	7.00
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20	0.20	0.20
*Premix	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.20	0.20	0.20	0.20	0.20	0.20
APSM	0.00	0.10	0.20	0.30	0.40	0.50
Total	**100**	**100**	**100**	**100**	**100**	**100**
Calculated Analysis:						
Crude protein	17.02	17.02	17.02	17.02	17.02	17.02
Crude fibre	4.16	4.15	4.14	4.14	4.13	4.11
Ether extract	3.68	3.68	3.68	3.67	3.67	3.66
Calcium	3.64	3.64	3.64	3.64	3.64	3.64
Metabolisable energy (Kcal/kg)	2702.30	2700.43	2698.00	2696.69	2694.82	2692.95

**Premix, vitamin-mineral premix (25kg/1000kg): contained vitamin A (10,000000IU), vitamin D3 (3,000000IU), vitamin E(30,000IU), vitamin K (2-3g), vitamin B1 (20g), vitamin B2 (5.0g), pyridoxine (3.0g), vitamin B12 (160mg), Biotin (60mg), Niacin (31g), Panthotenic acid (8g), Folic acid (1g), NMabgabese (85g), Zinc (50g), Iron (25g), copper (6g), Indine (1g) selenium (120g), Cpbalt (220mg), Antioxidant (125g), Choline chloride (200g). APSM = Alligator pepper (Aframomum melegueta) seed meal.*

Table 2: Haematological parameters (±SD) of laying birds fed graded dietary levels of *Aframomum melegueta* additive

Treatments	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**
Levels of APSM (%)	**0.0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**
Parameters:						
PCV (%)	32.03 ±2.61	30.13 ±1.90	31.27 ±2.70	31.53 ±1.69	31.93 ±1.23	32.47 ±3.18
Haemoglobin (g/dl)	11.43^{ab} ±0.06	11.97^{ab} ±0.92	12.50^{a} ±0.00	10.90^{b} ±0.00	11.97^{ab} ±0.92	10.90^{b} ±0.00
Red Blood cell ($x10^6/mm^3$)	2.43^{d} ±9.87	2.47^{d} ±0.21	2.80^{bc} ±0.17	3.13^{a} ±0.15	2.0^{cd} ±0.26	3.16^{ab} ±0.10
White Blood cell ($x10^3/mm^3$)	15.33 ±9.87	17.67 ±7.51	21.33 ±15.18	13.33 ±5.77	17.67 ±0.26	12.67 ±8.02
Platelets ($x10^3/mm^3$)	9.33 ±2.08	17.67 ±10.17	11.33 ±3.51	13.00 ±4.58	17.67 ±6.81	18.33 ±7.57
MCV (μ^3)	131.68^{a} ±11.04	123.13^{ab} ±17.45	112.21^{ab} ±15.20	100.65^{b} ±3.08	109.63^{b} ±2.13	104.63^{b} ±1.14
MCH (µµg)	46.94^{a} ±2.65	48.93^{a} ±7.50	44.75^{a} ±2.67	34.84^{b} ±1.67	119.36^{ab} ±16.81	35.19^{b} ±1.14
MCHC (%)	35.83^{ab} ±3.75	39.71^{a} ±1.71	40.18^{a} ±3.43	34.63^{ab} ±1.90	47.38^{a} ±4.09	33.78^{b} ±3.15

[a-d]Means with different superscript(s) across a row are significantly ($p<0.05$) different.
PCV = Packed cell volume; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration.

Table 3: Serum metabolites of laying Isa Brown birds fed graded dietary levels of *Aframomum melegueta* seed meal

Treatments	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**
Levels of APSM (%)	**0.00**	**0.10**	**0.20**	**0.30**	**0.40**	**0.50**
Parameters:						
Total protein (g/dl)	7.51^{a}	6.71^{b}	5.66^{f}	5.91^{d}	6.33^{e}	5.69^{e}
Albumin (g/dl)	6.92^{a}	3.96^{b}	3.25^{d}	3.24^{d}	3.25^{d}	3.49^{c}
Globulin (g/dl)	0.59^{f}	2.75^{b}	2.42^{d}	2.67^{c}	3.09^{a}	2.22^{e}
A/G ratio	11.61^{a}	1.45^{c}	1.33^{d}	1.23^{e}	1.05^{f}	1.58^{b}
AST (U)	290.58^{e}	335.58^{d}	341.84^{c}	354.42^{a}	341.34^{c}	344.24^{b}
ALT (U)	103.96^{f}	113.73^{c}	112.62^{d}	111.48^{e}	119.79^{a}	116.19^{b}
ALP (U)	90.81^{f}	336.47^{a}	137.32^{e}	176.09^{c}	116.42^{d}	183.26^{b}

[a-f]Means with different superscript across rows are significantly different ($p<0.05$).
A/G ratio = Albumin/Globulin ratio; AST = Aspartate Aminotranferase ; ALT = Alanine Aminotransferase; ALP = Alkaline Phosphatase.