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HAEMATOLOGY AND SERUM BIOCHEMICAL CHARACTERISTICS OF LAYING HENS FED DIETS CONTAINING BITTER LEAF (*Vernonia Amygdalina*) MEAL

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ABSTRACT

A feeding trial was conducted to determine the haematology and serum biochemistry characteristics of laying hens fed diets containing bitter leaf (vernonia amygdalina) meal. The bitter leaf was air-dried at room temperature and later milled. Four diets were formulated to contain 0, 1, 2 and 3 % inclusion levels of the BLM respectively. Each of the dietary treatments had four replicates, with ten birds per replicate, in a completely randomized design. Results showed that there was no significant ($p < 0.05$) difference between the PCV values of birds fed treatment 1 (37.40), 2 (36.87) and 3 (34.05). The results of the lymphocytes, heterophils, monocytes and eosinophils were not significantly ($P > 0.05$) influenced by bitter leaf meal compared with the control. Results of this study indicates that bitter leaf does not pose any health challenges to laying birds.

Introduction

In recent times, there has been a significant shortfall between the production and supply of animal protein to feed the ever-increasing population (Akorede et al., 2021) Poultry meat and eggs have been recommended among other animal products to bridge the protein gap because of their short generation interval, high rate of productivity, quick turnover rate, higher feed efficiency, low labour and land requirement (Gerber et al., 2015; Ojedapo et al., 2008).

The result of blood analysis is usually used to assess the health status of an animal (Okpe et al., 2022) An organism's physiology, nutrition, and pathological state can all be greatly impacted by the constitution of the blood, which provides information on the presence of many metabolites and other substances (Doyle, 2006). Haematological factors associated with blood and organs that generate blood, serve as reliable markers of an animal's physiological state (Khan & Zafar, 2005). According to Olafedehan et al. (2010), blood serves as a pathological reflector of an animal's condition after exposure to toxins and other factors. Thus, animals with healthy blood composition are likely to perform effectively (Okpe et al., 2016).

Numerous studies reported positive effects regarding the improvement of animal health and wellbeing after the administration of medicinal plants in animal nutrition (Mountzouris., 2011; Ghasemi et al., 2014; Kostadinović et al., 2015). The use of medicinal plants to feed laying hens have been reported to improve productive performance and also modulate cholesterol contents in egg (Kralik et al., 2022; Oueslati et al., 2020; Dauksiene, et al., 2020; Abdel-Wareth &

Lohakare., 2020) with benefits to consumers of eggs such as antioxidant properties (Kralik et al., 2022; Vlaicu et al., 2022; Vakili et al., 2022). One of such herbal plant is the bitter leaf plant which has been reported to be rich in proteins, fats, fibres, amino acids, minerals vitamins, and carbohydrates (Ali, Muazu, Diso & Ibrahim. 2020; Adeganmi, Adeganmi., Adeganmi & Oyekanmi (2020). Hence this study aimed to determine haematology and serum biochemistry characteristics of laying hens fed diets containing bitter leaf (*vernonia amygdalina*) meal.

Materials and Methods

Study location and experimental birds

The research work was carried out at the Poultry Unit of the Teaching and Research Farm of the School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology, Owerri. Imo State. The bitter leaf was procured from Relief market in Owerri, Imo State. They were air-dried at room temperature and later milled for subsequent inclusion at specified levels in the diets of growing pullets. A total number of one hundred and sixty birds were used for the experiment. Five birds were housed per cage measuring 66 x 49 x 45 cm and kept in a typical tropical poultry house with mean temperature of 27°C and relative humidity of 79 %. The birds were assigned to 4 dietary treatments T1, T2, T3 and T4 and 4 replicates with 10 birds each. All the layers in each cage were fed together and served as a replicate. Water was provided ad libitum from nipple drinkers fitted on top of the cages. All vaccination and other management practices were observed according to the conventional procedures. The experiment lasted for a period of 16 weeks. Four (4) experimental diets A, B, C and D were formulated which contained 0, 1, 2 and

3 percent inclusion of BLM respectively. The composition of experimental diets is shown in Table 1.

Data collection

At 15 weeks into lay, 5ml of blood was collected from veins around the wings of the thirty-two laying hens two birds per replicate into bijou bottles containing EDTA and stored under ice. These were taken to the Laboratory for haematological analysis. The red cell count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), total white blood cell count (WBC) and percentages of heterophil, lymphocytes and eosinophils were determined as described by Schalm *et al.* (1975). The mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated. 2 birds per replicate were selected and about 5ml of blood

samples was collected from the wing vein and put in bijou bottles without EDTA to produce serum for biochemical analysis. The blood samples were allowed to clot at room temperature and thereafter centrifuged at 2200 rpm for 15 minutes to separate serum from other cellular components and obtain clear serum for biochemical assays. The serum metabolites such as total cholesterol, triglycerides, high density lipoprotein and low-density lipoprotein were calculated. Glucose, creatinine, serum enzymes, electrolytes, total protein and reproductive hormones were also determined.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) using a completely randomized design. Means were separated using the Duncan's multiple range test.

Table 1 Ingredients composition of layers' mash containing bitter leaf meal

Ingredients	Inclusion level				
	0	1	2	3	4
Maize	54.00	54	54	54	54
SBM	20	20	20	20	20
PKC	5	5	5	5	5
Wheat offal	6	5	4	3	2
Fishmeal	4	4	4	4	4
Bone meal	4	4	4	4	4
Limestone	6	6	6	6	6
BLM	-	1	2	3	4
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Vit/Min*	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Calculated composition %					
ME(Kcal/kg)	2702.48	2676.4	2692.23	2651.28	2638.67
Crude protein	16.96	16.98	16.43	16.37	16.97
Ether extract	3.82	3.78	3.73	3.68	3.63
Crude fibre	4.03	4.03	5.13	4.16	4.23
Ash	3.16	3.19	3.13	3.17	3.14

*Vitamin and Mineral premixes ((TS-TOSAMVIT®, Hebei Junyu Pharmaceuticals Co., Ltd) formulated to provide per kg diet vitamin A-8,000,000 I.U; vit. D3- 1,6000,000 I.U, Vit. E-5,000 I.U; vit. K-2,000mg; thiamine-1,500mg; riboflavin-4,000mg; pyridoxine-1,500mg; niacin-15,000mg; vit. B12-10mg; pantothenic acid-5,000mg; folic acid-500mg; biotin-20mg; choline chloride-200g; antioxidant-125g; manganese-80g; zinc-50g; iron-20g; copper-5g; iodine-1.2g; selenium-200mg; cobalt-200mg.

Results and discussion

The result of the haematological indices of laying hens fed varying levels of bitter leaf meal is presented in Table 2. Among parameters measured, PCV, RBC, MCV, MCH, Platelets and WBC showed significant ($P<0.05$) differences among the means. Results showed that there was no significant ($p<0.05$) difference between the PCV values of birds fed treatment 1 (37.40), 2 (36.87 and 3 (34.05). This observation agrees with the findings of (Adu *et al.*

2020) who reported no significant differences in PCV, RBC, Hb and MCHC of broiler chickens fed clove. The findings also correlated with the research of Tang *et al.* (2017) who stated that the use of prebiotics and probiotics had no significant effects on haematological parameters (RBC, Hb, PCV, MCV and MCHC). Natural feed additives, as reported by (Oloruntola *et al.* 2019), supports normal haemopoietic processes of the laying hens. Although the PCV showed a decreasing trend as the quantity of

V. amygdalina increased across diets, the values were still within the normal range of 22% -35% (Mitruka and Rawskey, 1997) signifying that *V. amygdalina* does not have any deleterious effect on PCV values of layers.

Osho *et al.* (2014) demonstrated that oral administration of bitter leaf extract on broiler chickens did not have a significant effect on the Hb. The authors equally reported that blood parameters are the major indices of the physiological and pathological indicators of the nutritional status when compared to normal values. Changes in the constituent compounds of blood could be used to interpret the metabolic stage of an animal as well as the quality of the feed (Attia *et al.*, 2017; 2018; 2019). The higher values of these haematological indices in the birds administered *Vernonia amygdalina* leaf meal (VALM) may be due to their inability to cause haemolysis resulting from the anti-inflammatory potentials inherent in *Vernonia amygdalina* (Tokofai *et al.*, 2020). Therefore, the normal range of the values obtained in the present study indicates that the birds were not anaemic.

Variations in avian haematological blood values are a normal phenomenon, and depend in most cases on the physiological condition of the birds (Attia and Al-Harthi, 2015). Natural feed additives, as reported by (Oloruntola *et al.*, 2019), support normal haemopoietic processes of the laying hens. According to Isaac *et al.* (2013), PCV is involved in the transport of oxygen absorbed nutrients and also prevents anaemia.

The results of the lymphocytes, heterophils, monocytes and eosinophils were not significantly ($P > 0.05$) influenced by bitter leaf meal compared with the control. No significant differences were observed among the white blood cell differentials of the birds fed the diets supplemented with bitter leaf and the control diet. The various white blood parameters (lymphocytes, heterophils, monocytes and eosinophils) were within the normal range of haematological values for chickens which indicated that bitter leaf meal had no harmful effects on the immunity of the laying hen as reported by (Panda *et al.* 2008).

Table 2: Haematological Indices of Laying Hens fed varying levels of bitter leaf meal

Parameters	Inclusion levels of bitter leaf (%)				SEM
	0	1	2	3	
Hb (g/dl)	12.72	13.20	12.15	13.07	0.18
PCV (%)	37.40 ^a	36.87 ^a	34.05 ^a	24.57 ^b	1.88
RBC $\times 10^{12}/l$	2.20 ^{ab}	2.32 ^a	1.89 ^b	1.85 ^b	0.07
MCV (fl)	170.02 ^a	158.90 ^{ab}	182.32 ^a	127.50 ^b	7.50
MCH (pg)	57.85 ^b	56.90 ^b	64.55 ^{ab}	72.82 ^a	2.36
MCHC (g/dl)	34.02	35.95	35.80	63.00	4.48
Platelets $\times 10^9/l$	274.75 ^b	657.00 ^a	525.75 ^a	617.00 ^a	43.80
WBC $\times 10^9/l$	79.57 ^a	79.70 ^a	68.07 ^b	79.85 ^a	1.76
Lymphocyte (%)	84.50	80.00	82.25	80.75	1.14
Heterophil (%)	13.00	17.50	15.00	17.00	1.14
Monocyte (%)	1.25	1.25	1.25	1.00	0.19
Eosinophil (%)	1.25	1.25	1.50	1.25	0.20

*Mitruka and Rawsley (1977). Hb -Haemoglobin, PCV – Packed Cell Volume, RBC- Red Blood Cell, MCV- Mean Corpuscular Volume, MCH- Mean Corpuscular Haemoglobin, MCHC- Mean Corpuscular Haemoglobin Concentration, WBC- White Blood Cell

^{ab} Means in the same row bearing different superscripts are significantly different ($P < 0.05$)

The result of the serum biochemical indices of laying hens fed diets containing bitter leaf meal is shown in Table 3. No significant differences were observed among serum biochemical parameters of laying hens. Serum proteins are synthesized in the liver to maintain blood volume through the colloidal osmotic effect, buffer blood pH, transport hormones and drugs, cell coagulation, catalyse enzymatic reactions, regulate hormones, and defend body against foreign materials (Melillo, 2013). Alanine transaminase (ALT) are enzymes in the liver and are released when the liver is damaged, although there was a decrease in both with increasing concentration of BLM than the control, the levels of both in this study reflects normal liver function and shows that the liver was not damaged by

the bitter leaf meal inclusion in the diets. The reduction in BLM levels can be due to hepatoprotective effect of BLM which improves liver health. According to Onunkwo *et al.* (2021). Findings from this study does not suggest significant effects of BLM on the serum metabolites which indicated that bitter leaf does not pose any health challenges to the laying birds, especially as it relates to the liver (Obboh and Akindahunsi 2005). These research findings align with that of Al-Mufarrej *et al.* (2019), who observed no deleterious effects on the liver functions and immunity of broiler chickens fed diets supplemented with clove powder. Similarly, findings of Sharma and Choudhary (2017) reported that most medicinal herbs possess immune-boosting and anti-viral properties

Table 3: Serum Biochemical indices of Laying Hens fed varying levels of bitter leaf meal

Parameter	Inclusion levels of bitter leaf (%)				SEM
	0	1	2	3	
Total bilirubin (mg/dl)	0.41	0.36	0.43	0.41	0.01
Conjugated bilirubin (mg/dl)	0.11	0.09	0.15	0.14	0.01
Alanine transaminase (iu/l)	39.50	35.75	37.00	38.00	0.98
Alkaline phosphatase (iu/l)	516.50	458.75	478.50	455.75	10.39
Aspartate aminotransferase (iu/l)	44.00	48.25	43.00	45.00	0.90

Conclusion

The study concludes that inclusion of bitter leaf meal in the diets of laying hens did not affect haematological and serum biochemical values.

References

- Adu O.A, Gbore F.A, Oloruntola O.D, Falowo A.B. & Olarotimi O.J (2020) The effects of *Myristica fragrans* seed meal and *Syzygium aromaticum* leaf meal dietary supplementation on growth performance and oxidative status of broiler chicken. *Bull National Research Centre* 44:149
- Al-Mufarrej S. I, Fazea E.H, Al-Baadani H.H, Qaid M. M. (2019). Effects of clove powder supplementation on performance, blood biochemistry, and immune response in broiler chickens. *South African Journal of Animal Science*. <https://doi.org/10.4314/sajas.v49i5.6>
- Attia YA, Al-Harathi MA, Korish MM, Shiboob MM. Fatty acid and cholesterol profiles and hypocholesterolemic; atherogenic; and thrombogenic indices of table eggs in the retail market. *Lipids Health Dis*. (2015) 14:136. doi: 10.1186/s12944-015-0133-z
- Attia, Y. A., Al-Harathi, M. A., & Hassan, S. S. (2017). Turmeric (*Curcuma longa* Linn.) as a phyto-genic growth promoter alternative for antibiotic and comparable to mannan oligosaccharides for broiler chicks. *Revista mexicana de ciencias pecuarias*, 8(1), 11-21.
- Attia, Y.A, Al-Harathi, M.A & Elnaggar, A.S.H. (2018). Productive, physiological and immunological responses of two broiler strains fed different dietary regimens and exposed to heat stress. *Ital J Anim Sci*. 17:686–97. doi: 10.1080/1828051X.2017.1416961
- Attia, Y.A.; El-Naggar, A.S.; Abou-Shehema, B.M. & Abdella, A.A. (2019). Effect of supplementation with trimethylglycine (Betaine) and/or vitamins on semen quality, fertility, antioxidant status, DNA repair and welfare of roosters exposed to chronic heat stress. *Animals* 9, 547.
- Djakalia, B., Guichard, B. L., & Soumaila, D. (2011). Effect of *Moringa oleifera* on growth performance and health status of young post weaning rabbits. *Research Journal of Poultry Science*, 4(1), 7-13.
- Fotea, L., Costăchescu, E., Hoha, G., & Leonte, D. (2015). The effect of oregano essential oil (*Origanum vulgare* L.) on broiler performance. *Lucrări Științifice - Seria Zootehnie*, 53, 253-256.
- Ghasem, H. A., Kasani, N., & Taherpour, K. (2014). Effects of black cumin seed (*Nigella sativa* L.), a probiotic, a prebiotic and a symbiotic on growth performance, immune response and blood characteristics of male broilers. *Liv. Sci*, 164, 128 134.
- Goodarz, M., Nanekarani, S., & Landy, N. (2014). Effect of dietary supplementation with onion (*Allium cepa* L.) on performance, carcass traits and intestinal microflora composition in broiler chickens. *Asian Pac. J. Trop. Dis*. 4, S297-S301.
- Isaac, L.J., Abah, G., Akpan, B. & Ekaette, I.U (2013). Haematological Properties of Different Breeds and Sexes of Rabbits. *Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria*, pp. 24-27.
- Kostadinović, L. J., Lević, J., Popović, S., Čabarkapa, I., Puvača, N., Đuragić, O., & Kormanjoš, Š. (2015). Dietary inclusion of *Artemisia absinthium* for management of growth performance, antioxidative status and quality of poultry meat. *Arch. Geflügelk*, 79, 1-10.
- Melillo A. (2013). Applications of Serum Protein Electrophoresis in Exotic Pet Medicine. *Veterinary Clinics of North America Exotic Animal Practice*. 16(1):211–225
- Mitruka, H.M. and Rawnsley, S.K (1997). Chemical, Biochemical and haematological reference in normal experimental animal. *Mason, N.Y* pp. 287 -380.
- Mohammed, A. (2008). Pharmacognosy: pharmacognosy aqueous leaf extract of *Vernonia amygdalina* on lipoprotein and oxidative status in diabetic rat model. *Nigeria Journal of Physiological Journal of Natural Science Resources* 2 (7) pp.1-8.

- Mountzouris, K. C., Paraskevas, V., Tsirtsikos, P., Palamidi, I., Steiner, T., Schatzmayr, G., & Fegeros, K. (2011). Assessment of a phytogenic feed additive effect on broiler growth performance, nutrient digestibility and caecal microflora composition. *Anim. Feed Sci. Technol.* 168, 223 – 231.
- Oboh G. & Akindahunsi A. A (2005) Nutritional and toxicological evaluation of *Saccharomyces cerevisiae* fermented cassava flour. *Journal of Food Compos Anal* 8(7):731–738
- Okereke, C. (2003). Pesticidal properties of neem (*Azadirachta indica*), *Journal of Plant Science.*, 41, 97–99.
- Okpe, A. A., Abdullahi, J., Iyaode, I., & Ajagbe, A. D. (2022). Effect of Varying Dietary Levels of Bitter Leaf (*Vernonia Amygdalina*) Meal on the Serum Biochemistry of Finisher Broilers. *International Journal of Agricultural Economics, Management and Development*, 10(1), 162-170.
- Oloruntola, O.D., Ayodele, S.O., Adeyeye, S.A, Jimoh, A.O., Oloruntola, D.A & Omoniyi, S.I. (2019) Pawpaw leaf and seed meals composite mix dietary supplementation: effects on broiler chicken's performance, caecum microflora, and blood analysis. *Agroforestry System* 94:555–564. <https://doi.org/10.1007/s10457-019-00424-1>
- Onunkwo, D. N., Udeme, M. A., Ezea, J., & Ezenyilimba, B. N. (2021). Blood profile of finisher broiler chickens fed diet containing 8leaf meal composite as alternative to commercial broiler premix. *Nigeria Agricultural Journal*, 52(2), 306-311
- Osho, I. B., Akindahunsi, A., Igbasan, F. A., & Adekunle, D. J. (2014). Effect of orally administered bitter leaf (*Vernonia amygdalina*) extract on the growth performance and haematological parameters of broiler chicken. *Journal of Veterinary Medicine and Animal Health*, 6(10), 251-256.
- Panda, A.K., Kumar, A.A., Singh, S.D & Sastry V.R.B. (2008) Growth performance and pathological lesions in broiler chickens fed raw or processed karanj (*Pongamia glabra*) cake as a protein supplement. *Indian Journal of Animal Science* 78:997–1001
- Schalm, O. W., Jain, N. C., & Carrol, E. J. (1975). Normal values in blood of laboratory, fur bearing and miscellaneous zoo and wild animals, Pages 219 – 283. In: O. W. Schalm (Ed.) *Veterinary Haematology, 3rd edition*. Lea and Febiger, Philadelphia
- Sharma M. S & Choudhary P. R (2017). Effect of fenugreek seeds powder (*Trigonella foenum-graecum* L.) on experimental induced hyperlipidemia in rabbits. *Journal of Diet Supplementation*.14(1):1–8
- Sofowora, E.A (2008). Medicinal plants and traditional medicine in Africa. 3rd Edn., Spectrum Books Limited Ibadan, Nigeria, pp. 199-204.
- Tang S. G. H, Siew C. C, Ramasamy K, Saad W. Z, Wong H. K. & Ho Y. W (2017). Performance, biochemical and haematological responses and relative organ weights of laying hens fed diets supplemented with prebiotic, probiotic and symbiotic. *BMC Veterinary Research* 13:248
- Tokofai, M. B., Idoh, K., Oke, E. O., & Agbonon, A. (2020). Growth performance, haematological and biochemical parameters in broilers fed diets with varying levels of *Vernonia amygdalina* leaf meal. *European Poultry Science*, 84.