Physiological response of pullet chicks fed *Moringa oleifera*, *Ocimum gratissimum* and *Vernonia amgydalina* leaf meal-based diets

*Adetoro, B. O., **Olusola, O. O., **Iyayi, E. A., ***Olaniyi, T. A., ***Adedeyi, O. Y.,
***Popoola, M. A., ***Saka, A. A., ***Yahya, M. O. and ***Oyadeyi, O. S.

*Federal College of Animal Health & Production Technology, Moor Plantation, Ibadan,
Oyo State, Nigeria.

**University of Ibadan, Ibadan, Oyo state, Nigeria.

bcrown06@yahoo.co.uk 08052021759; olubunmiolusola@gmail.com

08161163668; eaiyayi@yahoo.com 08060721963

Abstract

Blood reflects the pathological status of animals exposed to toxicants and other conditions. Blood examination helps to clinically investigate the presence of metabolites and other constituents in the body of animals and plays vital roles in the physiological, nutritional and pathological status of animals. Blood constituents change in relation to the physiological status of an animal. A total of 144 one-day-old Isa-brown pullet chicks were randomly picked from a larger flock, weighed and distributed to groups with 4 replicates of 9 chicks each in a completely randomized design (CRD). Four feeds were formulated to meet the NRC requirement and allotted one each to the 4 group of birds. T1 was the control, T2, T3 and T4 contained 2% of *Moringa oleifera*, *Ocimum gratissimum* and *Vernonia amgydalina* leaf meals respectively. At the end of the experiment which lasted for 8 weeks 2 birds were randomly selected from each replicate for haematological and serum biochemical examination. All the haematological parameters measured were not significant ($P > 0.05$) except eosinophils while for serum biochemistry cholesterol and albumin to globulin ratio were significantly affected by dietary treatment ($P < 0.05$). The highest value (3.50 % eosinophils) was observed in the birds on diet 4 which had 2% of *Vernonia amgydalina* leaf meal and these birds also had the lowest (139.25 mg/dl) cholesterol value. It was therefore concluded that 2% of these leaf meals especially of *Vernonia amgydalina* can be incorporated in the diets of pullet chicks to reduce their blood cholesterol and boost their immunity.

Keywords: Haematology, Serum biochemistry, Pullet chicks, Leaf meals, Parameters,

Introduction

Blood is a vital special circulatory tissue which is composed of cells suspended in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis (Isaac *et al.*, 2013). Haematology refers to the study of the numbers and morphology of the cellular elements of the blood – the red cells (RBC), white cells (WBC), and the platelets (thrombocytes) with their use in disease diagnosis and monitoring (Merck Manual, 2012). Haematological studies function in diseases diagnosis and in investigating the level of damage to the blood (Onyeyili *et al.*, 1992; Togun *et al.*, 2007).

Haematological components, which consist of red blood cell (RBC), white blood cell (WBC) or leucocytes, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration are valuable in monitoring feed toxicity especially with feed constituents that affect the blood and of course the health status of farm animals (Oyawoye and Ogunkunle, 2004). RBC is a carrier of haemoglobin. It is this haemoglobin that reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration (Johnston and Morris, 1996; Chineke *et al.*, 2006). The major functions of the WBC and its differentials are to fight

infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. (Soetan *et al.*, 2013) and enhance adaptability to local environmental and disease prevalent conditions (Okunlola *et al.*, 2012; Isaac *et al.*, 2013). Feed components greatly influence blood parameters and especially now that so many unconventional ingredients are mostly included in livestock diets to reduce cost of production or improve the products obtained from such livestock to meet consumer's needs as they are mostly medicinal. The use of plant parts (roots, stems, leaves or a combination of one or more) in livestock feeds have been successfully carried out with great success. Anderson and Warnick (1989) have demonstrated the effectiveness of vegetables and indigenous leaf meals at reducing serum cholesterol in hypercholesterolemic humans. Although some of these leaf meals are high in protein and rich in minerals (Adeboye *et al.*, 2003), some of them contain anti-nutritional or toxic compounds. However, if these compounds can be removed, the resulting meals may be very good sources of plant protein that could serve as excellent animal feeds (Essiet and Solomon 2013). Some of the common meals are *Moringa oleifera* (drumstick leaf), *Ocimum gratissimum* (scent leaf) and *Vernonia amygdalina* (bitter leaf). Essential oils are present in these plants (Xu *et al.*, 2008) and antibacterial activities occur by reason of active substances in these essential oils (Cristani *et al.*, 2007). *Moringa oleifera* is a good source of vitamins, amino acids and has medicinal uses (Francis *et al.*, 2005) which have been used in the treatment of numerous disease conditions (heart disease and obesity) due to its hypo-

cholesterolemic property (Matthew *et al.*, 2001, Ghasi *et al.*, 2000). Oil from the leaves of *Ocimum gratissimum* has been found to possess antiseptic, antibacterial and antifungal activities (Edeoga, 2001). *Vernonia amygdalina* has been used as anti-malaria, purgative, anti-parasitic, treatment of eczema and for maintaining blood glucose levels (Nwanjo *et al.*, 2004). It is rather unfortunate that despite these properties and functions records are hardly available on the effect of the use of these medicinal plants on pullet chicks. Medicinal plants have contributed immensely to health care in Nigeria. This is due in part to the recognition of the value of traditional medical systems, particularly in Asian origin, and the identification of medicinal plant from indigenous pharmacopoeias, which have significant healing power. This project was therefore designed to evaluate the effects of using *Moringa oleifera*, *Ocimum gratissimum* and *Vernonia amygdalina* leaf meals on the blood parameters of pullet chicks.

Materials and methods

Test ingredients

Leaves from matured trees/ shrubs of *Moringa oleifera*, *Ocimum gratissimum* and *Vernonia amygdalina* were harvested at the vegetative phase and were air dried to constant weight hand-crushed and milled before incorporation into the diets.

Experimental diets

Chick mash was formulated to meet the specification of NRC 1994. T1 being the control diet, had no leaf meal, T2 contained 2kg *Moringa oleifera* leaf meal (MOLM)/100kg feed, T3 contained 2kg *Ocimum gratissimum* leaf meal (OGLM)/100kg feed T4 contained 2kg *Vernonia amygdalina* leaf meal VALM/100kg feed (chick mash) respectively.

Experimental design

A total number of 144 one-day-old ISA-brown pullet chicks were randomly picked, weighed and distributed to the 4 diets with 4 replicates of 9 chicks each, from a total of 357 one-day-old ISA-brown pullet chicks in a completely randomized design (CRD). They were mass brooded for 8 weeks in a well ventilated poultry house on deep litter. Water and feed were provided *ad libitum*. Routine vaccination was done according to the hatchery's recommendation. This experiment lasted for 8 weeks.

Data collection

Blood was collected at the end of the 8th week for haematological and serum biochemical examination, 2 birds per replicate were bled using hypodermic needle and syringe. Blood was drained into bottles pre-treated with EDTA, an anticoagulant and carefully labeled for haematological investigation. Packed cell volume (PCV) and Haemoglobin (Hb) were determined according to the method described by (Mitruka and Rawnsley, 1977). Erythrocyte count (RBC) and Leukocyte count (WBC) were determined according to the method described by Schalms *et al.* (1975). Blood constants: Mean Cell Volume, Mean Cell Haemoglobin and Mean Cell Haemoglobin Concentration were determined according to the method described by Jain (1986). Blood samples for biochemical indices were collected into other sample bottles containing no anticoagulant. These samples were spun in the centrifuge at 3,000 rpm

and the clearer portion decanted (after centrifugation) into small sample tubes stored in a freezer for biochemical investigations. Serum biochemical indices investigated include total protein, globulin, albumin, albumin- globulin ratio, creatinine, glucose, uric acid, cholesterol, triglycerides, high density lipoprotein (HDL), alanine phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino transferase (AST).

Proximate analysis

The test ingredients and the feed samples were analyzed using the methods recommended by Association of Official Analytical Chemists (A.O.A.C.1990).

Statistical analysis

Statistical analysis was carried out using one way analysis of variance of SAS (SAS, 1999) package. Duncan's multiple range test was used to separate significant means at 0.05

Results

The proximate analysis of the test ingredients is shown in Table: 1 in which the dry matter value ranged between 91.38% and 92.63%. The crude protein value ranged between 19.10% and 24.77%. The crude fiber value of *Moringa oleifera* leaf was 9.60 which is higher than that of *Ocimum grattissimum* leaf of 13.43% and *Vernonia amygdalina* leaf of 13.70%. The ash content of the test ingredients ranged from 3.78-7.23%. Ether extract ranged from 5.30% to 7.31% with 6.11%. The nitrogen free extract was 51.26% in *Moringa oleifera* leaf, 46.27% in *Ocimum grattissimum* leaf and

Table 1: Proximate composition of the test ingredients

Sample (%)	MOLM	OGLM	VALM
Dry matter	91.55	91.38	92.72
Crude protein	19.60	19.10	24.77
Crude fiber	9.60	13.43	13.70
Ether extract	7.31	5.30	6.28
Ash	3.78	7.28	7.23
Nitrogen free extract	51.26	46.27	40.75

40.75 % in *Vernonia amygdalina* leaf.

The result of the proximate analysis of the experimental feeds at the chick phase determined according to A.O.A.C. (1990) is shown in Table 2 in which the dry matter ranged between 91.55 to 92.19%. The crude protein content of the feed samples varied considerably between 18.90% –

22.05%. The crude fiber contents of the feed samples ranged from 7.10% - 20.40% which is considerably higher than the other diets. The ether extract of the feed samples ranged between (7.30 – 8.20%), ash content between (4.70 – 13.20%) and nitrogen free extract (23.99–46.40%).

Table 2: Proximate analysis of the experimental feeds at the chick phase (1-8 weeks)

Parameters (%)	T1	T2	T3	T4
Dry matter	92.19	92.10	91.80	91.55
Crude protein	18.90	22.05	21.15	19.25
Crude fibre	20.40	7.10	7.50	13.50
Ether extract	7.30	8.20	7.7	7.80
Ash	9.00	7.00	4.70	13.20
Nitrogen free extract	23.99	40.05	46.40	30.45

Table 3: Hematological and serum biochemical indices of the experimental birds at the chick phase (8weeks)

Parameters	Dietary Treatments				
	T1	T2	T3	T4	SEM
PCV (%)	27.75	25.87	27.37	28.25	1.55
Haemoglobin (g/dl)	9.21	8.10	8.93	9.05	0.55
RBC ($\times 10^6/\mu\text{l}$)	3.08	2.94	3.01	2.97	0.25
WBC ($\times 10^3/\mu\text{l}$)	17.51	16.54	16.48	16.48	1.11
Platelets ($10^3/\text{mm}^3$)	123.00	135.25	140.13	132.88	0.61
Lymphocytes (%)	66.25	68.375	64.87	67.50	1.99
Heterophils (%)	28.50	26.12	29.50	26.50	2.22
Monocyte (%)	2.87	2.25	3.25	2.37	0.42
Eosinophils (%)	2.12 ^b	2.87 ^{ab}	2.00 ^b	3.50 ^a	0.32
Basophils (%)	0.2	0.37	0.37	0.12	0.12
MCV(fl)	91.00	88.24	91.63	95.93	4.39
MCHC (%)	33.18	31.04	32.02	32.10	0.77
Total protein(g/dl)	5.13	4.87	4.56	4.55	0.21
Albumin (g/dl)	1.65	1.86	1.70	1.55	0.11
Globulin (g/dl)	3.16	2.68	2.83	3.00	0.15
AG ratio	0.51 ^{ab}	0.66 ^a	0.60 ^{ab}	0.47 ^b	0.05
AST(I.U./L)	168.00	149.75	144.88	168.25	12.60
ALT(I.U./L)	27.50	25.75	30.37	28.12	2.31
AP(I.U./L)	75.50	80.12	75.50	63.12	6.03
BUN(mg/dl)	0.21	0.23	0.30	0.23	0.04
Creatinine(mg/dl)	0.53	0.90	0.60	0.57	0.19
Cholesterol(mg/dl)	177.88 ^a	162.63 ^{ab}	152.75 ^{ab}	139.25 ^b	9.06
Glucose(mg/dl)	204.25	209.38	236.50	244.50	16.41
Triglyceride(mg/dl)	178.88	174.75	145.38	154.63	14.19
HDL(mg/dl)	67.75	62.75	57.87	70.37	5.30
LDL(mg/dl)	74.35	64.93	52.38	51.45	10.43

^{abc} Means within a row with different superscripts differs significantly ($P < 0.05$).

SEM- Standard error of the mean, PCV-Packed cell volume, RBC-Red blood cell, WBC-White blood cell, MCV-Mean Cell Volume, MCHC-Mean Cell Haemoglobin Concentration, MCH-Mean Cell Haemoglobin, AG ratio-Albumin Globulin ratio, AST-Aspartate amino transferase, ALT -Alanine amino transferase, Alanine phosphatase, BUN- Blood urea nitrogen, HDL-high density lipoprotein, LDL-low density lipoprotein

The haematology of the experimental birds at the 8th week (chick phase) is shown in Table 3 in which all the parameters measured were not significant ($P>0.05$) except the eosinophils in which the highest value of 3.50% was observed in the birds on VALM diet and the least value of 2.00% in birds on the OGLM diet. Variations in the values of other parameters had no particular trend. The serum biochemical parameters of the experimental birds at the 8th week were not significantly influenced by leaf meal ($P>0.05$) except for Albumin Globulin ratio and cholesterol. A:G was significant ($P<0.05$) with the highest value 0.66 MOLM and the lowest (0.47) in birds fed VALM based diets. Highest cholesterol value (177.88mg/dl) was obtained in birds on the control diets (no leaf meal inclusion) and least value (139.25mg/dl) in birds on VALM based diets.

Discussion

Of all the parameters measured for the three leaf meals, the eosinophils which is one of the components of the white blood cells that functions in conditions of allergy, asthma, hay fever, urticarial, parasitism and skin diseases (scabies, eczema, psoriasis, pemphigus) was significant ($P<0.05$). The highest value (3.50 %) was observed in the birds on diet 4 which had 2% of *Vernonia amygdalina* leaf meal it also had the lowest (139.25 mg/dl) cholesterol value, followed by those on diet 2 with a value of (2.87 %) observed in chicks fed 2% *Moringa oleifera* leaf meal and (162.63 mg/dl) cholesterol value, the next were those on the control diet with a value of (2.12 %) but it had the highest value (177.88 mg/dl) for cholesterol and the least value was observed in bird fed 2% *Ocimum gratissimum* (2.00 %) with (152.75 mg/dl) cholesterol. This is in

agreement with the report of Nworgu *et al.* (2013) that *Ocimum* reduced the blood cholesterol of growing pullets but some other parameters were significant in their study which were not significant in this work. This variation may be due to age difference of the pullets used. Though the normal blood parameters for pullet chick are yet to be well documented, Mitraka and Rawnsley (1977) reported a decrease in eosinophil count in acute infections and marked intoxication and since the leaf meals used contained some anti-nutrients (alkaloids, tannins, saponins, *etc.*), it's not surprising that there was an increase in the eosinophils observed in the blood of the chicks fed the leaf meals compared with the control diet. The cholesterol content of the birds fed diets containing the three leaf meals were also slightly reduced as the three leaf meals has been reported to hypocholesterolemic. The slight decrease could be as a result of cholesterol-reducing effects of the leaf meals in the diets which were probably due in part to the formation of insoluble complexes between cholesterol and the saponins present in the meals (Essiet and Solomon 2013). The formation of such complexes in the digestive tracts of the chickens would prevent the absorption of saponins (Essiet and Solomon 2013). Though albumin-globulin ratio was significant, it followed no particular order.

Conclusion

It can therefore be concluded that 2% of these leaf meals especially of *Vernonia amygdalina* can be incorporated in the diets of pullet chicks to reduce their blood cholesterol and boost their immune system. It was recommended that further researches should be conducted on pullet chicks in the tropics in order to have a database for blood parameters of tropical birds.

References

- Adeboye, O. C., Ogbé, O. F. D. and Barnidele, J. F. 2003** Ethnobotany: Indigenous leaf vegetables of South West Nigeria. *Delpinoa* **45**: 295-299.
- Anderson, J. O. and Warnick, R. E. 1989.** Sequence in which essential amino acids become limiting for growth of chicks and rations containing cotton seed meal. *Poultry Science Journal*, **45**: 84-89
- A.O.A.C. 1999.** *Official Methods of Analysis of the Association of Official Analytical Chemists*. Washington D.C
- Bamishaiye, E. I., Muhammad, N. O. and Bamishaiye, O. M. 2009.** Haematological parameters of albinorats fed on tiger nuts (*Cyperus esculentus*) tuber oil meal-based diet. *The International Journal of Nutrition and Wellness*, **10**(1). Retrieved from <http://ispub.com/IJNW/10/1/9293>.
- Chineke, C. A., Ologun, A. G. and Ikeobi, C. O. N. 2006.** Haematological parameters in rabbit breeds and crosses in humid tropics. *Pakistan Journal of Biological Sciences*, **9**(11), 2102-2106.
- Essiet, A. G. and Solomon, I. P. 2013.** Growth Performance, Carcass Characteristics, Lipid Profiles and Haematological Responses Of Broiler Finishers Fed With Leaf Meals Derived From 'Ntong' (*Ocimum gratissimum*) and 'Utasi' (*Gongronema latifolium*)” *IOSR Journal Of Pharmacy(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219* www.Iosrphr.Org Volume 3, Issue 8, Pp01-13
- Isaac, L. J., Abah, G., Akpan, B. and 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*. (p.24-27)
- Johnston, J. K. and Morris, D. D. 1996.** Alterations in blood proteins. In B. P. Smith (Ed.), *International Animal Medicine* (2nd edition.). USA: Mosby Publishers.
- Merck Manual 2012.** *Haematological reference ranges*. Merck Veterinary Manual. Retrieved from <http://www.merckmanuals.com/>.
- Mitruka, B. M. and Rawnsley, H. M. 1977.** *Clinical biochemical and hematological reference values in normal experimental animals* (p.134-135). USA: Masson Publishing Inc.
- National Research Council 1994.** Nutrient Requirements of Poultry. 9th Revised edition. National Academy Press, Washington DC.
- Okunlola, D. O., Olorunisomo, A. O., Aderinola, A. O., Agboola, A. S. and Omole, O. G. 2012.** *Haematology and serum quality of red Sokoto goats fed Baobab (Adansonia digitata) fruit meal as supplement to guinea grass (Panicum maximum). Proceedings of the 17th Annual Conference of Animal Science Association of Nigeria*. (p.427-433)
- Onyeyili, P. A., Egwu, G. O., Jibike, G. I., Pepple, D. J. and Ohaegbulam, J. O. 1992.** Seasonal variation in haematological indices in the grey-breasted guinea fowl (*Numida*

- mealagris Gallata pallas*). *Nigerian Journal of Animal Production*, 18(2), 108-110.
- Oyawoye, B. M. and Ogunkunle, H. N. 2004.** *Biochemical and haematological reference values in normal experimental animals*. New York: Masson. (p.212-218).
- Schalm, O. W., Jain, N. C. and Carroll, E. J. 1975.** *Veterinary haematology* (3rd edition, p.15-218). USA: Lea & Febiger, Philadelphia.
- Soetan, K. O., Akinrinde, A. S. and Ajibade, T. O. 2013.** *Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (Sorghum bicolor)* (p. 49-52). *Proceedings of 38th Annual Conference of Nigerian Society for Animal Production*. Vol. 2, Issue 1, 2014.
- Togun, V. A., Oseni, B. S. A., Ogundipe, J. A., Arewa, T. R., Hammed, A. A., Ajonije, D. C. and Mustapha, F. 2007.** *Effects of chronic Lead administration on the haematological parameters of rabbits – a preliminary study* (p. 341). *Proceedings of the 41st Conferences of the Agricultural Society of Nigeria*.

Received: 10th July, 2017

Accepted: 9th December, 2017