African Journal of Agriculture and Food Science
Volume 2, Issue 2, 2019 (pp. 17-26)

www.abjournals.org

HAEMATOLOGY, SERUM BIOCHEMISTRY AND LIVER ANTIOXIDANTS OF BROILER CHICKENS FED MORINGA LEAF MEAL SUPPLEMENTED DIETS AS A SUBSTITUTE TO DIETARY SOYBEAN MEAL

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ABSTRACT: *A four-week investigation was conducted to determine the influence of moringa (Moringa oleifera) leaf meal on the haematology, serum biochemistry and the liver antioxidant enzymes of broiler chickens at finishing stage. Two hundred birds were divided into 5 treatments and fed diets containing varying levels of moringa leaf meal (MLM) at the levels of 0, 12.5, 25, 37.5, and 50% to represent T1, T2, T3, T4 and T5 respectively. Each treatment group with 40 birds was further divided into 4 replicates of ten birds. The haematological variables of the chickens did not follow a particular trend and no significant ($p>0.05$) difference was observed in all the parameters measured but all were within the normal range documented for chickens which ranged as follows: haemoglobin (Hb) 8.15 to 9.75g/100, pack cell volume (PCV) 28.5 to 37.75%, red blood cells (RBC) ($10^6 mm^{-3}$) ranged from 2.84 to 4.09 and erythrocytes sedimentation rates (ESR), 2.50 to 4.38mm/hr. The serum biochemical indices indicated no significant ($p>0.05$) difference for total protein but there was a significant ($p<0.05$) effect in cholesterol with the highest content of 108.98g/dL found in T1 while the lowest, 93.64g/dL was recorded in T5. Serum albumin increased ($p<0.05$) with increase in MLM levels ranging from 2.90 in T1 to 4.70g/dL in T5. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also significantly ($p<0.05$) influenced by the diets. The results of liver antioxidant enzymes revealed that glutathione peroxidase (GTP) showed no significant ($p>0.05$) difference though superoxide dismutase (SOD) and catalase were significantly ($p<0.05$) affected by the dietary treatments. These results revealed that moringa supplementation in broiler finishers' diets have no adverse effects as shown in the haematological and biochemical parameters as serum cholesterol was significantly ($p<0.05$) reduced.*

KEYWORDS: Haematology, Broiler Chickens, Moringa Leaf, Dietary Soybean Meal, Serum Biochemistry, Liver Antioxidant Enzymes

INTRODUCTION

Haematology has been defined as the study of blood and its constituents which is an important part of clinical pathology as well as diagnostic process (Ologhobo *et al.*,2014). Blood is a bodily fluid in animals that delivers life sustaining substances inform of nutrients and oxygen to the cells and evacuates unwanted materials such as metabolic waste products out of the same cells to enhance normal functioning of cells, organs, tissues and the entire organism. In vertebrates, blood comprises of the cells suspended in fluid called plasma. Haematological profile in animals is an important indicator of their pathophysiological status

(Khan and Zafar, 2005). The assessment of the blood parameters in humans and livestock has been used overtime to measure the health status, diagnosis (Durai *et al.,* 2012) and to suggest curative techniques and perhaps further studies. Haematological variables are invaluable in monitoring feed utilization and toxicity in animal agriculture and related fields of study (Oyawoye and Ogunkunle, 1998). Hence, it can be ascertained that there is a correlation among what the animal consumes, its blood profile and health status. Haematological and biochemical profiles of livestock has been useful in monitoring the health status of livestock especially when fed with unconventional feed stuff (Hrubec *et al.*, 2000, Ologhobo *et al.*, 2014, Dienye and Olujimi, 2014). The serum is the plasma component of blood that is devoid of coagulation potential (Tijani *et al*., 2016). Blood plasma is a yellowish constituent of the blood that usually holds the blood cells in the whole blood in suspension. It is the fluid component of the blood that assists in conveying cells and nutrients throughout the body and it makes up about 55.8% of the body's total blood volume (Dennis, 1999). Serum is about 95% water that contains dissolved protein such as serum albumins, globulins and fibrinogen including glucose, hormones and electrolytes such as Na^{+}, Ca^{2+}, Mg^{2+}, $HC0_3^{-}$ and Cl^{-} and many others. Plasma plays significant roles in an intravascular osmotic influence that keeps electrolytes concentration balanced and insures the body from infections or haematological aberration. The phytochemicals in botanicals can influence feed utilization and blood components in livestock hence the need to monitor and measure haematological and serum biochemical indices of livestock when fed with unconventional botanical products. Antioxidants are certain group of vitamins, minerals, and enzymes that help to protect the body from the formation of free radicals (Imoru, 2018). They are substances which inhibit the oxidation of other molecules that are potentially damaging in the body and they protect cells from damage caused by unstable molecules known as free radicals by inhibiting initial free radical formation, preventing more production, chelating metals (pro-oxidants) and decomposing lipid peroxides (Ahn, 2016). Free radicals are atoms or groups of atoms that can cause damage to cells, impairing the immune system and leading to infections and various degenerative diseases such as heart disease and cancer. Free radical damage is thought by scientists to be the basis for the aging process. Nigerian soils support the abundant growth and good vegetative yield of *Moringa oleifera* however the direct consumption of its leaves and other component parts by humans has declined overtime despite several reported nutritional and health values of *Moringa oleifera* (Awar *et al.,* 2007, Olugbemi, *et al*., 2010 and Imoru *et al.,* 2018). High cost and competition between livestock and people for conventional protein sources such as soybean meal has also called for the need to employ leave protein in livestock feeding and management (Imoru *et al.,* 2019). One major indirect and alternative way of utilizing MLM for the benefits of humans is to ensure the incorporation of MLM in the diets of livestock. More so, the results of the effect of MLM on the haematological, biochemical and liver antioxidant parameters in broiler chickens have not been consistent, hence the need for the current study.

MATERIALS AND METHODS

Dietary Treatments and Management of Experimental Birds

The study was carried out at the Poultry Unit of the Teaching and Research Farms, Rufus Giwa Polytechnic, Owo, Ondo State and the birds were procured from a reputable hatchery in Ibadan, Oyo State, Nigeria which were initially raised in a deep litter system under good hygienic conditions. Two hundred 4 weeks old broiler chickens were subsequently divided into 5 treatment groups of 40 birds/treatment and 10 replicates/treatment. Soybean meal was partially replaced on equi-protein basis of 0, 12.5, 25, 37.5, and 50% with MLM to form dietary Treatments 1 (control), 2, 3, 4 and 5 respectively (Table 1).

Samples Collection and Analyses

At 4 weeks of the experiment, two birds per replicate were bled using sterile knives. Blood was drained into two different well labelled bottles for haematological and serum biochemical investigations. The blood samples for haematological parameters were collected into the bottle pre-treated with EDTA, an anticoagulant. Blood samples for biochemical indices were collected into another sample bottles containing no anticoagulant which were spurned in the centrifuge at 3,000 rpm and the clearer portion decanted (after centrifugation) into small sample tubes then stored in a freezer. Pack cell volume was determined by spinning about 75µl of each blood samples in heparinized capillary tube in a haematocrit centrifuge for about 5min and read on haematocrit reader (Benson *et al.,* 1989). Erythrocyte and Leucocytes counts were determined using Neubaur chamber method as described by Lamb (1981). The blood sample collected in each treatment was diluted at a ratio of 1: 200 for RBC counts using red cell diluting fluid while a dilution ratio of 1: 20 (blood: white cell diluting fluid) was used for WBC counts. Samples of RBC and WBC counts were obtained using the relationship:

RBC/µl = Numbers of red blood cells counted × 5 × 10 × 200

WBC/µ = Numbers of white blood cells counted × 0.25 × 10 × 20.

Haemoglobin was estimated using cyanomethaemoglobin method. 0.02 ml of blood was expelled into 4 ml drakin's solution. The mixture was allowed to stand for 5min for full colour development. Sample haemoglobin concentration was obtained using this relationship:

$$\text{Sample haemoglobin} = \frac{\text{Reading of Standard haemoglobin concentration (g/100ml)}}{\text{Reading of standard}}$$

Table 1: % Composition of Experimental Diets

	Equi- Protein Replacement of SBM with MLM, %				
	0	12.5	25	37.5	50
Maize	53	53	53	53	53
Maize offal	8	7.64	7.58	7.48	7.38
Groundnut cake	12	12	12	12	12
Soybean meal	16	14	12	10	8
Palm kernel cake	7	6	4.7	3.44	2.18
Moringa leaf meal	0	3.36	6.72	10.1	13.4
Bone meal	2.8	2.8	2.8	2.8	2.8
Limestone	0.3	0.3	0.3	0.3	0.3
Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Salt	0.4	0.4	0.4	0.4	0.4

Composition of premix per kg of feed: *Vitamin A (E-672) = 4 800 000 IU, Vitamin D3 =1 200 000 IU, Vitamin E = 6 000mg,Vitamin K3 =1 200mg, Vitamin B1=400 mg, Vitamin B2=2 000 mg, Vitamin B6=1 200 mg, Vitamin B12=8 mg, Folic acid=400 mg, Niacin=12 000 mg, Pantothenic acid=2 800 mg, Choline chloride =240 000 mg, Iron (E-1) (Ferrous =10 000 mg, Iodine =120 mg, Cobalt = 80 mg, Copper=2 400 mg, Manganese = 40 000 mg, Zinc =20 000 mg, Selenium =50 mg, dl-Methionine=80 000 mg, Ethoxyquin (antioxidant = 40 000 mg, Biotin=28 mgandCarrier ad=1 000 g.*

The haemoglobin (Hb) concentration and the blood constants: mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean cell volume were determined using cyanomethaemoglobin method and appropriate formula used respectively (Jain, 1986):

$$MCH = \frac{\text{Haemoglobin content (g/100g)}}{\text{RBC count milion/cu. mm}} \times 10 \text{ or } MCH = \frac{\text{Haemoglobin content (g/L)}}{\text{RBC count } (10^{12}/L)} \times 10 \text{ or}$$

$$MCHC = \frac{\text{Haemoglobin content (g/100ml)}}{\text{PCV}} \times 100$$

The smear of each blood samples in the bottles containing EDTA was made on a clean slide, air dried and fixed in methanol for three minutes. The smear was stained in Giemsa stain for 30 mins, rinsed in water and air- dried. The cell count for each sample was carried out as described by Lamb (1981). During the process, the slide was viewed under the microscope

and cells were counted. From the cell counted, the percentage of different cells (neutrophils, eosinophil, lymphocytes and monocytes) were determined.

Serum biochemical indices investigated were total protein, globulin, albumin and cholesterol, alanine amino transferase (ALT) and aspartate amino transferase (AST). The serum total protein was determined by the biuret method using a commercial kit (Randox Laboratories Ltd, U.K), while albumin value was obtained by bromocresol green method. Also, the cholesterol was determined by nonane extraction and enzymatic colorimetric methods, respectively using commercial kit (Quimica Clinica Applicada, S.A), while the serum enzymes; ALT and AST were obtained using the Randox Laboratories Ltd, UK test kits.

To determine the liver antioxidative enzymes, liver tissue (5g) was taken from slaughtered birds in each replicate prior to scalding using dissecting knife in a sterile condition and the antioxidants measured using the methods described by Ismail *et al.* (2012).

RESULTS

Mean values of haematological indices of broiler chickens are shown in Table 2. There was no significant ($p>0.05$) difference in all parameters measured. The parameters ranged as follows: haemoglobin, 8.15 to 9.75g/100, PCV, 28.50 to 37.75%, RBC ($10^6 mm^{-3)}$, 2.84 to 4.09 and ESR, 2.50 to 4.38mm/hour. The lymphocytes constitute the highest proportions of the WBC and varied from 59.38 to 63.38%. The MCHC, MCH and MCV had their range also as follows, 33.31 to 34.12%, 21.03 to 42.58pg and 92.11 to 127.22µm respectively. The results of serum biochemical indices and liver antioxidant parameters of broiler chickens are presented in Table 3. There was no statistical ($p<0.05$) difference in total protein but a numerical difference exists ranging from 3.26 in T1 to 3.78 g/dL in T5. However, a significant ($p<0.05$) difference was recorded in serum cholesterol content of birds which indicated a continuous decrease as dietary MLM increased (Figure 1). Birds in T1 had the highest value (108.98g/dL) while those in T5 obtained the lowest value (93.64g/dL). Serum albumin increased with increase in dietary MLM levels ranging from 2.90 to 4.70g/dL for broiler chickens in T1 and T5 respectively. Globulin was relatively stable across diets and showed no significant ($p>0.05$) difference. ALT and AST had a similar pattern of increase with increasing MLM levels in the diets. Result of liver antioxidant enzymes revealed that glutathione peroxidase showed no significant ($p>0.05$) difference across treatments. Superoxide dismutase (SOD) in the livers of birds was significantly ($p<0.05$) influenced by the diets. An increase in the level of MLM inclusion brought about a corresponding increase in SOD. Catalase (CAT) values ranged between 0.10 to 0.19mol/g tissue with T1 having the least and T4 obtaining the highest ($p<0.05$) score.

Table 2: **Haematological Indices of Broiler Finishers Fed Graded Levels of Moringa Leaf Meal Supplement Diets**

	Moringa leaf meal inclusion levels, %						
	0	12.5	25	37.5	50	**SEM**	***P***
Haemoglobin (g/100)	9.75	9.58	8.15	8.53	8.91	3.21	1.66
Packed cell volume (%)	29.25	28.75	28.5	35.	37.75	5.11	1.11
Red blood cells (10^6mm^{-3})	2.9	2.84	2..9	3.8	4.09	7.23	1.12
Erythrocyte sedimentation. rate (mm/hr)	2.5	2.5	4.38	4.38	3.5	1.22	0.69
Lymphocytes (%)	59.38	61.13	62.25	63.13	63.38	2.12	0.91
Heterophils (%)	25.25	23.38	23	20.75	20.63	4.17	0.1
Monocytes (%)	12.5	12.5	12	13.38	13	2.11	1.34
Basophils (%)	2.13	2	2	2	2	0.41	0.01
Eosinophils (%)	0.75	1	0.75	0.75	1	0.1	0.01
Mean cell haemoglobin conc. (%)	33.33	33.32	33.27	34.12	33.31	6.27	0.14
Mean cell haemoglobin (pg)	42.58	33.73	28.1	22.45	21.03	3.87	1.19
Mean cell volume (pm)	127.22	101.23	98.28	92.11	92..3	6.33	1.88

[abc] *Mean along the same row without common superscript differ at p>0.05*

Table 3. Serum Biochemical Indices and Liver Antioxidants of Broiler Chickens Fed Graded Levels of Moringa Leaf Supplemented Diets

	Moringa leaf meal inclusion level, %						
	0	12.5	25	37.5	50		
Serum Biochemical Indices						**SEM**	***P***
Cholesterol (mg/dL)	108.98^a	106.23^a	105^a	100.88^b	93.64^c	2.27	0.67
Total protein (g/dL)	3.26	3.46	3.54	3.67	3.78	3.01	1.11
Albumin (g/dL)	2.9^b	3.29^a	4.05^a	4.58^a	4.7^a	1.2	0.69
Aspartate (g/dL)	59.49^b	59.71^b	70.14^a	67.03^a	79.35^a	8.11	0.91
Alanine (g/dL)	62.81^b	89.06^a	84.73^a	78.93^a	89.78^a	10.16	0.1
Globulin (g/dL)	0.37	0.16	0.52	0.61	0.92	0.11	0.01
Liver Antioxidants (mol/g tissue)							
Glutathione peroxidase	2.22	2	1.86	1.88	2.01	0.1	0.01
Superoxide dismutase	32.37^c	43.48^b	50.48^a	49.28^a	51.75^a	3.17	0.14
Catalase	0.1^c	0.14^b	0.17^a	0.19^a	0.15^b	0.1	0.01

[abc] Mean along the same row without common superscript differ at p>0.05

DISCUSSION

All the haematological values reported in the present research, including the Packed Cell Volume, Red Blood Cell, Haemoglobin, Erytrocytes Sedimentation Rate, Heterophils, Monocytes, Eosinophils, Lymphocytes, Mean Cell Haemoglobin Concentration, Mean Cell Haemoglobin and Mean Cell Volume were within the physiological ranges reported for broiler chickens (Mitruka and Rownsley, 1977 and Ross *et al.*, 1978). These results are suggestive that nutritional and chemical compositions of MLM are not in any way detrimental or inhibitory to blood formation process and possess no blood anti-metabolites. Haematological characteristics of livestock suggested their physiological disposition to the plane of nutrition (Maduake and Ekenyem, 2006). Haematological components of blood are also valuable in monitoring feed toxicity especially with novel test ingredients that may likely affect blood formation (Oyawoye and Ogunkunle, 1998). The biochemical parameters of this work revealed that serum cholesterol drastically reduced with increase in the MLM inclusion levels as birds fed control diets (T1) had the highest serum cholesterol content while those in T5 (MLM 50%) had the least cholesterol level. This observation agrees with the reports of Ghasi et al. (1999) and Ewuola et al. (2011) who reported that crude extracts from moringa leaves possess hypocholesterolemic potency even at very low dosage. Albumin increased with increase in MLM levels. Serum glucose and globulin were relatively stable across diets and showed no significant ($p>0.05$) difference. Alamine aminotransferase (ALT) and Aspertate aminotransferase (AST) had a similar pattern of increase with increasing MLM levels in the diets. Increasing levels of serum total protein, globulin and albumin are beneficial to the animals and indicative of nutritional advantage imposed by the test ingredient (MLM) in the diets over the control. Though relative stable levels of ALT and AST indicate the hepato-protective effect of the test ingredient of the feeds while their elevation along dietary increase of test meal is suggestive of hepatic cellular damage leading to their leakage into blood circulation (Mousa *et al.*, 2008). The current results are not in agreement with those reported by Ologbobo *et al.* (2014) that recorded no significant ($p>0.05$) effect in serum biochemical parameters of broiler birds fed MLM as a substitute for oxytetracycline. Low level of substitution (0.20-0.50%) might be responsible for the non-significant influence reported.

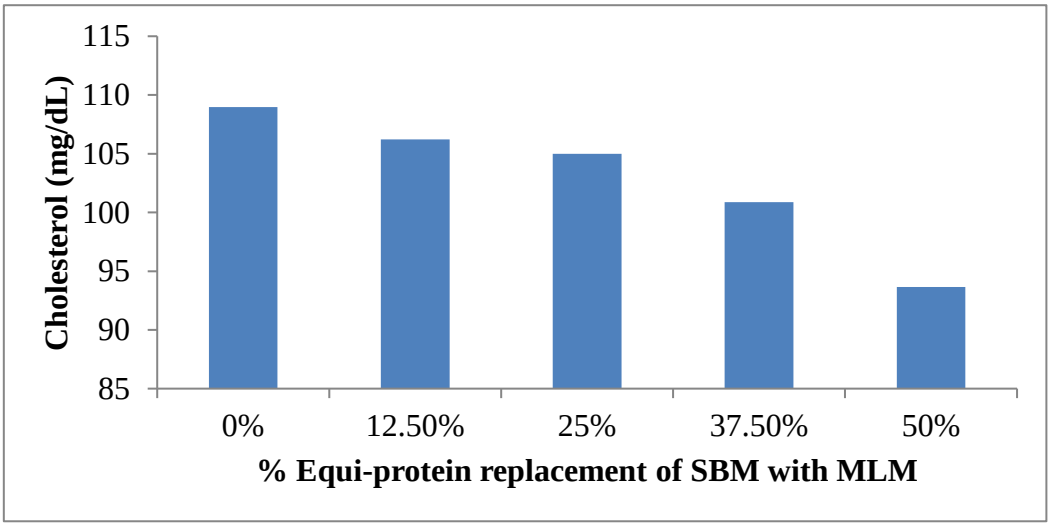

Figure 1. Effects of Substituting Soya Bean Meal with Moringa Leaf Meal on the Serum Cholesterol Level

Antioxidants are groups of vitamins, minerals, enzymes or phenols that protect the body from the formation or demobilizing already formed free radicals. They inhibit the oxidation of other molecules that are potentially damaging to the body. The increase in liver antioxidant enzymes catalase (CAT) and superoxide (SOD) is a confirmation that MLM is very rich in antioxidants that can be made bio-available to animals through diets to enhance performance and product quality. Antioxidant enzymes can easily be accessed in the liver, kidneys or the erythrocyte. This result agrees with the affirmations of Onibi and Osho (2007) and Onibi *et al*. (2009) that herbs contain potential antioxidants and antimicrobial agents that are beneficial to broiler chickens.

CONCLUSION

The outcome of the current study indicates that MLM showed no detrimental influence on the haematological, serum biochemical and liver antioxidant parameters of broiler chickens even up to 50% replacement of SBM protein. Moringa leaf meal having hypocholesterolemic potential, can be useful in reducing serum cholesterol in livestock. It may therefore be recommended as a good leaf protein substitute to soybean protein.

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