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National Animal Production Research Institute
Ahmadu Bello University
P.M.B 1096, Shika-Zaria,
Kaduna State, Nigeria.
Email:japr@napri.gov.ng Url:journals.napri.gov.ng

PHYSIOLOGICAL RESPONSE OF BROILER CHICKENS FED REPLACEMENT LEVELS OF *Moringa oleifera* LEAF AND MAIZE OFFAL

[*1]Bah, U. J., [2]Baburo, A. B., [1]Obianwuna, U. E. and [3]Mohammad, A. A.
[1]Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Animal Science, Faculty of Agriculture, Bayaro University Kano, Nigeria
[3]*Department of Animal Science, University of Maiduguri, Maiduguri, Nigeria*
[*]*Corresponding Author: usmanjibrinbah@gmail.com +2348026164046*

ABSTRACT

A study was carried out to evaluate the haemato-biochemical parameters of broiler chickens fed diets containing proportions of maize Offal (MO) and Moringa oleifera leaf (MOL) at Teaching and Research Farm of Bayero University Kano. A total of one hundred and fifty day old broiler chicks (Arbor Acre) were used for the experiment. They were fed on super starter vital feed during one week physiological adjustment period. Thereafter, the birds were randomly selected and allocated to five dietary treatment groups. Each treatment contained thirty (30) birds and replicated 3 times with ten (10) birds per replicate in a Completely Randomized Design (CRD). The experimental diets were T_1 (30%MO/0.0%MOL), T_2 (22.5%MO/7.5%MOL), T_3 (15.0%MO/15.0%MOL), T_4 (7.5%MO/22.5%MOL) and T_5 (0.0%MO/30%MOL). At the end of 56 days feeding trial, six birds were randomly selected from each treatment group, two per replicate for haematological and serum biochemical analysis. The results showed that there was no significance ($p>0.05$) difference in haematological parameters except Monocyte ($p<0.05$), platelets ($p<0.05$) and means corpuscular haemoglobin ($p<0.05$) while plasma urea and cholesterol were also affected ($P>0.05$) among the treatment means in serum biochemical profile. Although, variation observed among the treatment means but fell within the reference values for healthy bird. Based on the results obtained from the study, it revealed that MOL can be used as partial replacement up to 22.50% without adverse effect on health status of broiler chickens

Keywords: Moringa oleifera leaf, Maize offal, Broiler, Haemato-biochemical parameters

INTRODUCTION

Poultry industry in the developing countries is facing some challenges, one of which is an increase in the cost of feed because of high prices of protein and energy sources (Abbas, 2013). These have compelled researchers to direct their attention to non-conventional feed resources with particular emphasis on cereal grain substitutes. The use of leguminous multipurpose trees and shrubs has been suggested to be a viable alternative source of proteins, vitamins and minerals for poultry feeding. Plant leaves are commonly processed into leaf meals for use as poultry feed. Plants leaf meals which have been widely used in feeding non- ruminant animals include; Leucaena leucocephala, Gliricidia sepium, Sesbania sesban and Manihot esculenta (Gadzirayi *et al*., 2012). However, Moringa oleifera is presently being focused globally as another promising leaf meal in livestock feeding. It is drought-tolerant and grows at a rainfall of 250-1500 mm per year (Martin, 2007). Presently it is among those plants that have not been studied for many years but now is being investigated for its fast growth, higher nutritional value and increasing utilization as a livestock fodder crop (Nouman *et al*., 2013). The feeding value of Moringa oleifera has been

re-counted to be analogous to that of soybeans and rapeseed meal (Soliva *et al*., 2004). With the leaves of Moringa being rich in nutrients, pregnant women and lactating mothers use the powdered leaves to enhance their children's nourishment, principally in under developed countries where malnutrition is common (Stephen *et al*., 2008 and Sudha *et al*., 2010).

Haematology has been defined as the study of blood and it plays an important role in clinical pathology as well as in disease diagnostic process (Lutz and Prytulski 2008). It is used not only in the examination of the cellular and fluid portions of blood, but also in the study of tissues that form, store and circulate blood cells.

Serum or plasma is the liquid component of blood which lacks coagulation factors. It is similar to interstitial fluid in which the correct composition of key ions that act as electrolytes is essential for normal functioning of muscles and nerves. Other components in the serum include proteins, which assist with maintenance of pH and osmotic balance while giving viscosity to the blood; antibodies, or specialized proteins that are important for defense against viruses and bacteria; lipids, including cholesterol, which are transported in the serum; and various other substances including nutrients, hormones, metabolic waste and external substances, such as drugs, viruses and bacteria (Martina, 2007).

Haematological and serum analysis is usually used to assess the health status of an animal. It has been observed as good indicators of the physiological status of animal and its changes are important in assessing the response of animal to various physiological situations (Khan and Zafar, 2005) while biochemical parameters assess general nutritional status and to identify specific nutritional deficiencies (Brugler, 2002). In view of this, study was carried out to assess the effects of substitutional level of feeding Moringa oleifera leaf on haematological and serum biochemical profile of broiler chickens.

MATERIALS AND METHODS

Experimental Site

The study was conducted at Bayero University Teaching and Reasearch Farm, Kano. Kano lies within Latitudes $12^{o}25^{'}N$ and $12^{o}40^{'}N$ and Longitudes $8^{o}35^{'}E$ and $8^{o}45^{'}E$. The climate is characterized by four seasons: the hot-dry season, the warm-wet season, the warm-dry season and the cool-dry season. The annual average temperature is $27\pm7^{o}C$ (Olofin and Tanko, 2002). Rainy season lasts for about five months, with average annual rainfall of 1000mm at the southern part which gradually decrease to about 800mm northward (Ezenekwe *et al.*, 2013).

Experimental Birds and their Management

A total of 150-day-old broiler chicks (Arbor Acre) were used for the experiment. The birds were provided with a warm environment to the temperature of $36\text{-}38^{o}C$, and fed on super starter vital feed during one week adjustment period. Deep litter house partitioned into pens that accommodated 10 to 15 birds were used. Vaccines against infectious diseases like infectious bursal disease and Newcastle disease were given. On day 12th, Gumboro was administered through their drinking water. On the 15th day, Lassota was administered through their drinking water. On the 19th day, second Gumboro was administered to them. Coccidiostat drug was administered through drinking water from 23rd-27^{th} day. On the 35th day, second lassota was administered. The experimental diets and clean drinking water were supplied *adlibitum* to the birds. Adequate ventilation was also ensured throughout the experimental period, which lasted for 56 days.

Experimental Diets and Design

Five experimental diets were formulated using dried *Moringa oleifera* leaf, maize, soybean meal, groundnut cake, maize offal, wheat offal, bone meal, fish meal, lysine, methionine and premix in such a way that *Moringa oleifera* leaf (MOL) was substituted for maize offal (MO) as T_1 (30%MO/0.0%MOL),

T_2 (22.5%MO/7.5%MOL),
T_3 (15.0%MO/15.0%MOL),
T_4 (7.5%MO/22.5%MOL) and
T_5 (0.0%MO/30%MOL).
The feeds were subjected to proximate analysis using standard analytical methods of AOAC (1990), table 1 and 2. Birds were randomly selected, weight and allotted to five (5) dietary treatment groups of 30 birds each and replicated three times with 10 birds per replicate in a completely randomized design.

Table 1: Composition of Broiler Starter Diets

	Diets (%)				
Ingredients	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
Maize	35.00	35.00	35.00	35.00	35.00
Maize Offal	0.00	7.50	15.00	22.50	30.00
Moringa oleifera	30.00	22.50	15.00	7.50	0.00
Wheat Offal	9.00	8.00	8.00	7.00	6.00
SBM	15.00	16.00	16.00	17.00	18.00
Fish Meal	5.00	5.00	5.00	5.00	5.00
Bone Meal	4.00	4.00	4.00	4.00	4.00
Premix*	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
Methionine	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00
Calculated Nutrients Analysis					
Crude Protein (%)	22.22	22.29	22.02	21.91	20.55
Ether Extract (%)	4.34	4.11	3.94	3.75	3.38
Crude Fibre (%)	5.45	5.31	5.51	5.39	5.45
Lysine (%)	1.02	1.07	1.14	1.16	1.18
Methionine (%)	0.97	1.09	1.05	1.13	1.24

*: Vitamin A 10,000,000i.u; vitamin D_3 200,000i.u; vitamin E 23,000mg; vitamin K_3 2,000mg; vitamin B_2 5,500mg; Niacin 27,500mg; Pantothenic acid 7,500mg; vitamin B_6 3,000mg; vitamin B_{12} 15mg; Folic acid 750mg; Biotin 60mg; Choline chloride 3,000,000mg; Cobalt 200mg; Copper 3,000mg; Iodine 1,000mg; Iron 20,000mg; Manganese 40,000mg; Selenium 200mg; Zinc 30,000mg; Antioxidant 1,250mg. Avitech Health LTD. Batch C.

Table 2: Composition of Broiler Finisher Diets

	Diets (%)				
Ingredients	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
Maize	35.00	35.00	35.00	35.00	35.00
Maize Offal	0.00	7.50	15.00	22.50	30.00
Moringa oleifera	30.00	22.5	15.00	7.50	0.00
Wheat Offal	11.00	10.00	10.00	8.00	8.00
SBM	14.00	15.00	15.00	17.00	17.00
Fish Meal	4.00	4.00	4.00	4.00	4.00
Bone Meal	4.00	4.00	4.00	4.00	4.00
Premix*	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
Methionine	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00
Calculated Nutrient Analysis					
Crude Protein (%)	18.86	18.67	18.20	18.17	18.01
Ether Extract (%)	4.85	4.34	3.82	3.57	3.34
Crude Fibre (%)	5.94	5.82	5.73	5.77	5.69
Lysine (%)	0.77	0.82	0.93	1.01	1.08
Methionine (%)	0.77	0.85	0.85	0.93	1.01

*: Vitamin A 10,000,000i.u; vitamin D_3 200,000i.u; vitamin E 23,000mg; vitamin K_3 2,000mg; vitamin B_2 5,500mg; Niacin 27,500mg; Pantothenic acid 7,500mg; vitamin B_6 3,000mg; vitamin B_{12} 15mg; Folic acid 750mg; Biotin 60mg; Choline chloride 3,000,000mg; Cobalt 200mg; Copper 3,000mg; Iodine 1,000mg; Iron 20,000mg; Manganese 40,000mg; Selenium 200mg; Zinc 30,000mg; Antioxidant 1,250mg. Avitech Health LTD. Batch A

Data Collection

Data were collected on Chemical analysis, haematological and serum biochemical parameters during period of the experiment

Chemical Analysis

Data for the MO and MOL were analyzed using standard analytical methods of AOAC (1990) to determine dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), whereas nitrogen free extract (NFE) and metabolizable energy (ME) were calculated using the formula of Pauzenga (1985). ME (Kcal\kg) = 37 x %CP + 81.8 x %EE + 35.5 x %NFE.

Haematological and Biochemical Studies

Blood samples were collected at the end of the trial from two birds per replicate and six birds per treatment. The birds were bled in the morning between 06.30 and 09.00 h to avoid excessive bleeding.4ml were collected from the wing vein using a sterile disposable needle after sterilizing the collection site with antiseptic Oyewole *et al*. (2017). Samples for hematological evaluation were collected into

ethylene diamine tetra acetic acid (EDTA) treated tubes to prevent coagulation. Red blood cell (RBC), white blood cell (WBC), haemoglobin (HB), packed cell volume (PCV), monocytes, lymphocytes and neutrophils counts were determined as described by Ewuola and Egbunike (2008). Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration were determined using appropriate formulae as described by Emiola *et al*., (2013) whereas serum biochemical parameters, namely; total protein, albumin, globumin, cholesterol, platelates, urea and creatinine were determined using methods described by Bahman *et al.* (2011). Chlorine, Sodium and Potassium ions by flame photometry Schales and Schales (1941).

Statistical Analysis

Data obtained from the experiment were subjected to One-way Analysis of Variance (ANOVA), using General Linear Model (SAS, 2001). Significant differences among means were separated using Least Significant Difference (L.S.D.) test at P<0.05 (SAS, 2001).

RESULTS AND DISCUSSION

Table 3: Proximate Composition of *Moringa oleifera* Leaf Meal and Maize Offal

Parameters	MOLM (%)	MO (%)
Dry Matter	92.60	89.61
Organic Matter	91.81	92.87
Crude Protein	28.64	9.37
Crude Fibre	8.47	12.31
Ether Extract	5.82	4.65
Ash	8.23	5.13
Nitrogen Free Extract	43.94	58.54
M.E(kcal/kg)	2493.41	2761.43

MOLM: Moringa oleifera Leaf Meal, MO: Maize Offal, ME: Metabolizable Energy

Haematological Parameters of Broiler Chickens Fed Varying Proportions of Moringa oleifera leaf meal and Maize offal in Diets

The results of haematological parameters are shown on Table 4. There were no significant (P>0.05) difference among the treatments except for monocytes (p<0.05), platelets (p<0.05) and Mean corpuscular haemoglobin (p<0.05). The difference of monocytes could be the reason in trying to convert foreign bodies as broiler chickens experience unset of respiratory ailment at finisher phase. Monocytes are known to inflame tissues and undergo hypertrophy to form large active phagocytic monocytes which play role of engulfing and destroying foreign bodies in the blood (Abdu, 2014). The significant (p<0.05) difference of platelate indicated the influence of the test diets as *Moringa oleifera* leaf is highly reputed for its positive effect on haematological variables (Chinwe and Isitua, 2010). Platelets are well known for their vital role in the maintenance of homeostasis (George, 2000) and release a vast array of bioactive chemicals such as homeostatic factor and cationic protein (Elzey *et*

al., 2005). Although significant (P<0.05) difference in MCH but fell within the normal ranges of 33 to 47pg (Jain, 1993). It could be the positive effect of test diets since it is the estimate haemoglobin per unit red blood cell.

Table 4: Haematobiochemical Parameters of Broiler chicks Fed Varying Proportions of MOLM and Maize Offal in Diets

Parameters	T_1	T_2	T_3	T_4	T_5	LSD
PCV (%)	38.80	35.33	37.33	35.77	32.37	8.261
WBC ($\times 10^3/\mu L$)	2.86	2.40	2.74	2.49	2.42	0.68
RBC ($\times 10^6/\mu L$)	2.86	2.40	2.74	2.49	2.42	0.68
Lymphocytes (%)	55.33	53.00	60.33	57.00	57.33	15.69
Neutrophils (%)	35.00	37.00	29.67	36.67	35.00	16.59
Monocytes (%)	9.67^{a}	9.00^{ab}	6.67^{ab}	6.00^{b}	6.67^{ab}	3.25
Platelet $(\times 10^3 \mu/L)$	4.67^{b}	6.00^{b}	5.00^{b}	26.00^{a}	11.00^{ab}	17.53
Haemoglobin *(g/dL)*	11.80	10.37	11.37	10.87	9.60	2.95
MCV *(fL)*	136.20	143.47	137.00	143.63	133.97	11.32
MCH *(Pg)*	41.20^{bc}	43.17^{ab}	41.47^{bc}	44.20^{a}	39.67^{c}	2.34
MCHC *(g/dL)*	30.30	30.10	30.27	30.40	29.60	1.77

PCV= Packed Cell Volume, WBC= White Blood Cell, RBC= Red Blood Cell, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin, MCHC=Mean Corpuscular Haemoglobin Concentration, a, a,b, ab = Means on the same row with different superscript are significantly different

The result of serum biochemical analysis is shown on Table 5. There were no significant (p>0.05) differences among the parameters across treatments except for the urea (p<0.05) and cholesterol (p<0.05). This is similar to the report of Aderinola *et al*. (2013) who obtained significant (P<0.05) higher values of urea in broiler chickens fed 0, 0.5, 1.5 and 2% MOLM. Iyayi and Tewe (1998) reported that serum urea and total protein values depend on the quantity and quality of dietary protein. The concentrations of cholesterol in this experiment were within the values of 84.94 to 192.66mg/dL reported by Meluzzi *et al*. (1992). Cholesterol is nutritionally the key intermediate for the synthesis of many sterols while higher level of cholesterol is associated with increased risk of cardiovascular diseases in chickens.

Table 5: Serum Biochemistry of Broiler Chickens Fed Various Proportion of MOLM and Maize Offals in Diets

Parameters	T1	T2	T3	T4	T5	LSD
Albumin (*g/dL*)	2.19	4.14	1.79	4.58	3.35	4.22
Globulin(*g/dL*)	13.69	10.40	10.47	15.33	8.14	19.824
T. Protein(*g/dL*)	15.97	14.54	12.27	19.90	11.49	22.289
Creatinine(*mg/L*)	0.50	0.74	0.54	0.64	0.50	0.267
Urea(*mmol/L*)	1.03^{a}	0.80^{ab}	0.30^{b}	0.40^{b}	0.73^{ab}	0.562
Cholesterol (*mg/dL*)	117.67^{ab}	100.00^{b}	101.33^{b}	100.00^{b}	126.67^{a}	20.933
Potassium (*mmol/L*)	5.37	7.00	5.37	6.70	4.89	4.10
Sodium (*mmol/L*)	156.67	165.00	176.00	115.67	153.67	67.847
Chlorine (*mmol/L*)	98.00	97.33	95.00	102.00	106.00	48.02

A, b, ab =Means on the same row with different superscript are significantly different,
LSD = Least Significant Difference, MOLM = Moringa oleifera leaf meal

CONCLUSION

From the results obtained in this study, it is concluded that the higher the replacement level of MOLM in diets of broiler chickens the more the adverse effects on cholesterol. Broiler chickens can tolerate up to 22.50 % level of MOLM in the diet without adverse effects on their health status. Therefore, it is recommended for the farmers.

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