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Effect of Dietary Levels of *Moringa oleifera* Seed Meal on Growth, Haematological and Biochemical Profiles of Broiler Finisher Chicken

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Abstract: Consumption of saturated and trans fats can elevate blood cholesterol levels thereby increasing the risk of heart disease. Excessive fatty deposit associated with broiler meat due to ad libidum feeding has been reported to affect the quality of the processed chicken. There has been reports that different herbs and natural products are effective in lowering serum cholesterol levels in animals thereby producing leaner and healthier broiler meat. In view of recent concerns on effects of bad lipids on human health and claims that the supplementation of broiler diets with *Moringa oleifera* seed meal might positively affect the carcass quality, this study was initiated. This trial evaluated the effects of feeding undecorticated *Moringa oleifera* seed meal on growth performance, haematological and biochemical values of broilers finisher birds. The air dried seeds were milled in a hammer mill to produce *Moringa oleifera* Seed Meal (MOSM). Four broiler diets were formulated to contain the MOSM at 0, 8, 12 and 16%, respectively and were used to feed 96 broiler finisher birds. The birds were divided into 4 groups and randomly assigned to the 4 treatment diet with to as control diet and T_1, T_2, T_3 as the Moringa treatment diet in a Completely Randomized Design (CRD) with 3 replicates per treatment. Water and feed were provided ad libitum. At the end ofn the experiment, six birds per treatment were randomly selected for collection of blood samples for haematological analysis. Blood samples were collected and dispensed into tubes containing Ethylene Diamine Tetra Acetic acid (EDTA) as anticoagulant and another set into tubes without coagulant for serum analysis. At the end of the 7 week experimental period, results indicated that Average Final Body Weight (AFBW) and Average Daily Weight Gain (ADWG)

decreased significantly ($p<0.05$) with increased inclusion of *Moringa oleifera* Seed Meal (MOSM), although, T_2 showed least value in ADWG. The results showed that haematological indices such as packed cell volume, red blood cells, mean cell volume, mean cell haemoglobin, haemoglobin were not significantly ($p>0.05$) influenced by varied levels of MOSM and were within the normal range (9.06-31-0×106/g) recommended for broiler birds except for WBC, lymphocyte, neutrophiles and platelets which were higher than the average values. The results of the biochemical parameters indicated that urea was significantly ($p<0.05$) influenced by increasing levels of MOSM compared to the control. However, elevated values of serum cholesterol, sodium, chlorine, glucose and calcium were observed, although, not significant. All other serum indices were not significantly ($p>0.05$) influenced by increasing levels of MOSM. The study suggests that the dietary inclusion of MOSM significantly reduced weight gain but did not reduce the serum cholesterol levels in the broiler birds.

INTRODUCTION

Nigeria's forest is rich in nutritionally and medicinally important flora[1]. Among such plants is *Moringa oleifera* commonly called horseradish tree or drumstick tree and locally known as Zogeli among the Hausa speaking people. Almost every part of *M. oleifera* can be used as food and feed for livestock. The leaves can be eaten fresh, cooked or stored as dried powder for several months while he pods when fresh can be cooked or stored as dried powder. *Moringa oleifera* seed oil and micronutrients have been reported to contain antitumor, antiepileptic, antidiuretic, anti-inflammatory and anti-venom properties. The oil is highly valued by perfumers for its power of absorbing and retaining odours and by watch makers as a lubricant[2]. Moringa seed cake obtained as a byproduct of pressing seeds to obtain oil is used to filter water using flocculation to produce portable. water for animal or human consumption . The oil cake is used as a fertilizer. Moringa seeds contain dimeric cationic proteins which absorb and neutralize colloidal charges in turbid water causing the colloidal particles to clump together making the suspended particles easier to removes.

Moringa oleifera plant extracts possess some antioxidant compounds and nutrients (carbohydrates, proteins and lipids) which are essential requirements for chicken growth[3]. These antioxidant properties are reported to be safe and have received remarkable attention due to the ability to preserve foodstuffs and prevent rancidity caused by oxidation[4].

The plant has also been advocated for traditional medicines for centuries[5]. Antimicrobial action of moringa seed meals have been validated after the discovery of inhibitory activity against several micro organisms. In a recent study, aqueous extracts of *Moringa oleifera* seed was found to be inhibitory against many pathogenic bacteria including *staphylococcus aureus*, *Baciilus subtilis*, *Escherichia coli*. Moringa seed extracts were also found to have inhibitory action against *Mycobacterium phlei* and[6]. Leaf extract of moringa was found to be effective in checking growth of fungi *Basidiobolus haptosporus* and *Basidiobolus ranarums*[7]. Besides antibacterial activity of moringa oils it also possesses anti-fungal activity[8]. A study showed that moringa seed extracts has an antimicrobial activity against *Streptococcus*, *Salmonella typhi*, proteus mirabilis[9]. Anthonine found in moringa roots has potent in inhibitory activity against *Vibrio cholera*[7].

Besides the medicinal benefits of moringa seed meal, the high protein content make it a potential replacement for soybean meal or fish meal where it is economical to do so and if there is absence of anti-nutritional factors which could interfere with the normal process of metabolism. The crude protein content of the seed meal has been reported to range from 16-40%. In addition, they are reported to be good sources of minerals such as calcium, magnesium, potassium, iron as well as vitamins A, C and E, carotenoids, flavonoids and selenium, making it an antioxidant[4, 5, 10]. Despite the reported properties of moringa, the utilization of the seed meal as feed additive for broiler finisher birds has not been widely reported, especially, around the South East zone of Nigeria. In addition, information on their effect on growth, heamatological and biochemical parameters of broiler chickens is scanty.

There have been reports that different herbs and natural products are effective in lowering serum cholesterol levels there by producing leaner and healthier broiler meat[11-13]. Excessive fatty deposit associated with broiler chickens during ad libidum feeding may reduce the quality of the processed chicken. In view of recent concerns on effects of bad lipids in the human health[14], the supplementation of *Moringa oleifera* seed meal in broiler diets might positively affect the carcass quality by reducing abdominal fat accumulation[15]. This is because moringa contain tannins, alkaloids, saponnins and inhibitors which bind to cholesterol, thus, hampering its absorption in the intestine. Furthermore, as a natural antioxidant, moringa seeds can extend the shelf-life of meat products and are considered to be safer than other synthetic antioxidants. This is

possible because dietary vitamin E has been reported to reduce or prevent the process of lipid oxidation (major cause of quality deterioration in meat). This study was therefore, designed to evaluate the effect of *M. oleifera* seed meal on growth, heamatological and biochemical profiles of broiler finisher chicken.

MATERIALS AND METHODS

Experimental site: The experiment was conducted at the Federal University of Technology Owerri Teaching and Research Farm, Ihiagwa, Imo-state, South Eastern Nigeria between September and October, 2014. It is located in latitude 5.485°N and longitude 7.035°E in the humid rain forest zone of Nigeria, high temperatures occur during the months of November-April with average maximum temperatures occurring in February and March while the lowest are experienced in July.

Sources of feed ingredients and experimental animals: Commercial feeds were purchased from local feed dealers in Owerri Imo-state at the starter phase. The various feedstuff used in formulating the experimental diets at the finisher phase were also purchased from local feed dealers within Owerri. The day-old chicks were purchased from Zion Livestock Enterprises, Owerri, Imo-state. Moringa seed meal was obtained from an established moringa plot around the World Bank housing Estate Owerri, Nigeria as well as a plot at the FUTO Teaching and Research Farm.

Processing of moringa seeds: The harvested moringa seeds were air dried a shed until they were crispy to touch, then milled using a hammer mill to a particle size of about 3 mm to obtain a product herein referred to as Moringa Seed Meal (MOSM) which was stored in sacs until needed.

Procurement of birds and experimental design: A total of 103 day-old unsexed broiler chicks (B-not) were used in this experiment. On arrival chicks were given water and measured amounts of feed were placed on the feed trays up to 10 days of age. The chicks at day-old weighed between 50-55 g. Optimum temperature was maintained using cooking stoves and lanterns for the first three weeks and the pens were covered with black polythene. The polythene sheet were uncovered during hot days to allow for sufficient air exchange, since, the birds are now able to regulate their body temperature. All the chicks were housed in the same pen during the brooding stage but at the end of the brooding period, four dietary treatments, T_1, T_2, T_3 and T_4 containing graded levels of MOSM were prepared, containing *Moringa oleifera* seed meal at 0, 8, 12 and 16 kg in 100 kg of mixture, respectively with. T_1, designated as the control diet. The birds were housed separately in a 12 pen partition and fed separately in a deep litter system. The experiment was a completely randomized design. Each treatment was replicated three times with eight birds each, making a total of 24 birds per treatment giving a total of ninety-six birds. Each treatment was fed the designated diet during the finisher phase beginning at 4 weeks of age for 2 weeks until the birds were 7 weeks old.

Data collection: A pre-weighed diet was fed *ad libitum* each day and the left over feed weighed at the beginning of the next day. The difference between the amount fed and the left over fed represented the quantity of feed consumed. Average feed consumption in a treatment was obtained by dividing the weight of feed consumed in that treatment by the number of birds in that treatment group expressed in grams. The following parameters were measured in the course of the 14 day trial, namely; initial weight, final body weight, weekly body weight gain, average daily weight gain, average daily feed intake and feed conversion ratio.

Determination of live weight gain/loss: The birds were weighed at the beginning of the experiment and at the end of each week and the weights recorded. The average weight per treatment was obtained by dividing the total weight of birds in the, respective, treatment group by the number of the birds in that treatment group.

Determination of feed conversion ratio: The feed conversion ratio for body weight gain was calculated as follows:

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

Blood collection and laboratory analysis: At the end of the feeding trial three birds were randomly selected for each treatment (1 bird per replicate for blood sample collection). The blood was collected from the bronchial vein under the wings of the birds. The blood sample of about 2.5 mL in a 5 mL sterilized syringe was collected per bird for the biochemical value analysis and another blood sample was collected and transferred into a universal bottle containing EDTA for haematology. This was done to prevent the blood sample from clotting and EDTA serves as an anticoagulant. It preserves the blood for a specific time depending on the parameter and mixed thoroughly to ensure that the blood does not settle down to give a wrong result upon analysis.

Haematology indices: The following haematological indices were determined, Haemoglobin (HB), Packed Cell Volume (PCV), Red Blood Cell (RBC), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), Platelets (PLT), White Blood Cell (WBC), Lymphocyte (LYM), Monocyte (MONO), Neutrophile (NEUT).

Serum biochemical indices: The following biochemical indices were determined urea, creatinine, totalbilirubin, albumin, protein, glucose, cholesterol, calcium, phosphate, Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Bicarbilurin.

RESULTS AND DISCUSSION

The results of the performance parameters are shown in Table 1-3. The Analysis of Variance (ANOVA) showed that there were significant differences ($p<0.05$) in all parameters evaluated except for Average Daily Feed Intake (ADFI) which showed no significant difference ($p>0.05$).

Average final body weight: The initial body weight showed significant differences ($p<0.05$) which was highest for T_2(775 g) followed by T_1(756 g), T_0(737.5 g) and lowest in T_3(687.5).

This could be due to inherent variabilities in feed consumption and inability to balance the weights of birds at the start of the experiment. The Average Final Body Weight (AFBW) and Average Daily Weight Gain (ADWG) decreased significantly ($p<0.05$) with increased inclusion of *Moringa oleifera* Seed Meal (MOSM), although, T_2 showed least value in ADWG. The considerable decline in average daily weight gain with increased moringa seed meal inclusion could be due to the presence of some anti-nutritional factors which limits the digestibility and utilization of diets containing MOSM irrespective of its high nutritional status. This agrees with what Kieg and Fox reported that anti-nutritional factors like phenols and saponins or tannins could interfere with the normal protein metabolism by binding nutrients present in feeds thereby decreasing availability and utilization.

Also, phytate reduces bioavailability of minerals in non ruminant animals and decline digestibility of starch and protein. It has also been reported that growth performance could be reduced if the inclusion level of *Moringa oleifera* seed meal is above 5-10% in broiler diets[13].

Table 1: Composition of the experimental finisher diet dietary level of Moringa oleifara seed meal

Feed materials	D1	D2	D3	D4
Maize	55	55	55	55
Moringa seed meal	-	8	12	16
Soya bean meal	25	17	13	9
PKC	5	5	5	5
Wheat offal	10	10	10	10
Oyster shell	1	1	1	1
Bone meal	3	3	3	3
Salt	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.5
Lysine	0.25	0.25	0.25	0.25
Total	100	100	100	100

Table 2: Calculated chemical composition of experimental diet

Parameters	T1	T2	T3	T4
Crude protein	19.35	18.24	17.68	17.12
Crude fibre	4.18	5.61	6.81	7.69
Ether extract	3.35	4.50	5.08	5.66
ME (Kcal/Kg)	2859.45	2644.44	2536.94	2429.43

Feed intake: The result show a significant decrease ($p<0.05$) in Average Daily Feed Intake (ADFI) with increased inclusion level of MOSM from T_0(144.05) through T_1(135.42), T_3(126.19) to T_2(111.90).

Although, there were no significant differences between, T_1 and T_3 ($p>0.05$) and between T_2 and T_3, they all differed significantly from the control showing that inclusion of the moringa seed meal depressed feed intake ($p<0.05$). This could be due to reduced palatability of the diets as also reported by[16]. Also, Omekam[17] observed that unpalatability nature of a feed stuff will consequently prevent chickens from consuming adequate quantities of feed.

The Feed Conversion Ratio (FCR) of diets containing MOSM (T_1, T_2, T_3) were significantly similar ($p>0.05$) but significantly higher than the control (T_0). This suggests that birds fed the control diet produced the highest quantity of meat for each quantity of feed consumed. When compared to the other diets containing moringa. This agrees with the report that increased CF, EE and Ca in poultry diet due to MOSM inclusion hinders protein and energy digestibility and depresses feed intake as well as the enzymatic activites that promotes carbohydrate, protein and fat digestion[18].

Haematological values of broiler birds fed treatment diets: The result obtained from the Analysis of Variance (ANOVA) shows that every parameter measured in haematology and serum biochemistry except for urea were similar ($p>0.05$). The result from Table 4 shows that every of the parameter except White Blood Cell (WBC), Lymphocytes (LYM), Nuetrophiles (NEUT) and Platelates (PLT) which were high were within the normal

Table 3: Performance of broiler finisher birds fed graded levels of moringa oleifera seed meal (mosm) (gram)

Parameters	T_0	T_1	T_2	T_3	SEM
Average initial body weight	737.50^{c}	756.50^{b}	775.00^{a}	687.50^{d}	3.169
Average final body weight	1283.33^{a}	1037.50^{b}	962.50^{c}	933.33^{c}	26.352
Average Weight gain	545.83^{a}	281.00^{b}	187.50^{c}	245.83^{bc}	25.846
Average Daily weight gain	77.97^{a}	40.14^{b}	26.78^{c}	35.12^{bc}	3.694
Average daily feed intake	144.05^{a}	135.42^{ab}	111.90^{c}	126.19^{bc}	9.673
Feed Conversion Ratio	1.86^{a}	3.37^{b}	4.39^{b}	3.59^{b}	0.581

SEM- standard error mean; abc- means with different superscript along the same horizontal row are significantly different ($p< 0.05$) NB T_0, T_1, T_2 and T_3 are treatment diets containing 0, 2, 3 and 4% of MOSM, respectively

Table 4: Effects of graded level of Moringa oleifera seed meal on the haematological values of finisher broilers

Parameters	T_0(0%)	T_1(8%)	T_2(12%)	T_3(16%)	SEM
HB (g dL^{-1})	9.03^{ab}	9.23^{b}	9.03^{ab}	5.00^{a}	1.772
PCV (%)	27.73	28.57	28.43	24.97	2.924
RBC ($X10^6$/UL)	2.24	2.33	2.29	2.09	0.257
MCV (fl)	123.93	122.73	124.17	119.63	2.558
MCH (pg)	30.4	39.63	39.40	38.3	3.917
MCHC (g dL^{-1})	32.57	32.27	31.70	28.03	2.919
PLT ($X10^3$/UL)	67.00	63.00	66.67	70.67	9.062
WBC ($X10^3$/UL)	61.13	68.03	66.80	65.50	5.937
Lym (%)	88.33	87.00	84.33	81.00	5.926
NEUT ()	11.67	13.00	15.67	19.00	5.926

$^{a, b}$means with different superscript are significantly different ($p<0.05$) within rows

range and reference interval given by Mitruka etc. The values of Haemoglobin (HB), Packed Cell Volume (PCV) and Red Blood Cell (RBC) of the diets containing moringa at different levels were higher than the control diet (0%), although, it decreased with increasing moringa level except in T_3(16%) which showed a drastic drop in HB and PCV below the control (0%) and the established range.

The result of PCV followed a similar pattern with HB, indicated that the diets T_2 and T_3 were nutritionally adequate to meet the protein needs of the birds. Lower HB and PCV concentration in T_3(16%) apart from indicating signs of anaemia, may also suggest that the birds had low protein intake which coincided with lower feed intake[19].

The same trend in HB, PCV and RBC applied to WBC and MCH from T_1-T_3 (16%), the values did not fall below that of the control (0%). MCHC and lymphocyte decreased from T_0-T_3 with increasing moringa inclusion from 8-16% but the reverse was the case for NEUT and PLT, PLT. First, it decreased from T_0-T_1 and then increased with higher inclusion level of *Moringa oleifera* seed meal. These increases above normal reference range of WBC, LYMPH, NEUT and PLT may be an indication of a challenge in health condition within the body. In the case of PLT. White Blood Cells (WBCs) are responsible for protecting the body against infections and consist of lymphocytes, monocytes, neutrophils, eosinophils and basophils. They amongst other functions attack Foreign bodies in the blood such as virus-infected cells, enhance the production of antibodies and engulf Foreign materials (antigens). A higher presence could therefore, connote a threat to normal health and the body builds up its defense against such threat. Although, *Moringa oleifera* has been claimed to boast immune system[11, 20] it is however, most likely that such properties are contained and restricted to the pod which possesses lectin (substance that modulates the body defense system). The general non-significance of the WBC across treatments in this trial may be indicative of the fact that t the diets neither impaired nor enhanced the birds ability to wade off infection.

Biochemical values of broilers birds fed treatment diets: The results from Table 5 showed that every of the parameters except for ALP, protein, glucose and calcuim were within the reference range and interval given by Bounous etc. This is an indication that the diets were nutritionally adequate to meet most of the body need of the birds. The result showed a significant decrease in urea from T_0-T_3 with increasing moringa level with reduced crude protein content of the diet. Also an increasing protein and globulin this according to reports could be dependent on the quality and quality of protein supplied in the diet. Infact, Esonu etc.. reported that low serum urea and high total serum protein is an indication of good quality protein utilization. The numerical mean differences in Na, K, CL, BICARB, calcium and phosphate which relatively increased could be as result of nutrient availability and utilizability due to moringa inclusion or due to water availability for proper digestion and absorption of the diet's nutrients.

Table 5: Effects of Graded Level of Moringa oleifera Seed Meal on the Biochemical Values of Finisher Broilers

Parameters	T0	T1	T2	T3	SEM
Urea	12.40[b]	12.13[b]	8.63[a]	8.43[a]	1.132
Creatinine	0.107	0.103	0.123	0.127	0.035
Na C	137.93	149.20	141.90	143.55	5.041
K	4.6	4.47	4.19	4.67	0.576
CL	103.90[a]	125.37[b]	117.40[ab]	120.03[ab]	8.459
BICARB	27.33	28.33	26.67	27.67	1.027
PH	6.91[ab]	6.85[ab]	6.94[b]	6.82[a]	0.041
TB	0.09	0.08	0.07	0.38	0.220
Cb	0.05	0.04	0.04	0.03	0.009
Ast	15.33	14.33	14.00	13.33	4.637
ALT	6.33	4.67	3.67	4.67	1.972
ALP	943.50[a]	1685.63[b]	1397.83[ab]	1421.73[ab]	231110
Protein (g dL^{-1})	3.1	3.2	3.07	3.07	0.69
Albumin (g dL^{-1})	1.7	1.6	1.8	1.7	0.319
Glucose (mg dL^{-1})	139.1[a]	154.27[a]	155.87[a]	220.43[b]	23.991
Cholesterol (mg dL^{-1})	103.93	140.17	114.97	141.13	25.897
Calcium (mg dL^{-1})	12.79	15.88	15.11	14.55	1.506
Phosphate (mg dL^{-1})	6.27[ab]	6.8b	6.6[ab]	5.37[a]	0.613
Globulin (g dL^{-1})	1.4	1.6[q]	1.27	1.7	0.690

a and b means with different superscript are significantly different ($p<0.05$) within rows

This reflects what Iheukwumere and Herbert reported that water restriction significantly influences the biochemical constituents (Na, CL, HCO_3, Ca) in the sera of the kidney. AST and ALT were significantly similar ($p>0.05$) and fell within range, decreasing with increasing moringa in diet. ALP showed significant differences ($p<0.05$) with T_1 having the highest value but similar to T_2 and T_3 and, although, significantly different from T_o. The values of ALP were above the reference range given by Bahman Abdi-Hachesco. This could be as a result of high pH as indicated in Table 5 which was slightly reduced by increasing moringa inclusion. It could also be due to the metal lowering action of substances such as phytates found in moringa seeds. Abnormal high blood levels of alkaline phosphate may indicate disease in bone or liver, bile duct obstruction or certain malignant diseases[21]. The cholesterol trend in the experiment which showed a numerical elevation or increment is not in agreement with Ewuola *et al*.[22] who reported that crude extract from moringa leaves was found to be a potent hypocholesterolaemic agent. It may mean that the potency of the hypocholesterolaemic activity is limited to the leaves alone. The cholesterol value of the group fed without MOSM diet were lowest. It was also observed that the cholesterol content increased with increased MOSM inclusion this may also result in high fat content in the meat. In other studies, animals fed diets rich in cholesterol or saturated fat had elevated blood cholesterol levels.

CONCLUSION

From the result of the study, the inclusion of *Moringa oleifera* Seed Meal (MOSM) at different levels does not to have a beneficial effect on broiler growth and biochemical profile of broiler chickens. The could be because of the anti-nutritional factors contained in the seed meal despite the reported high protein and other nutrients profile.

RECOMMENDATIONS

Further studies are recommended that would remove the anti-nutritional factors by processing in order to harness its potential as an alternative feed ingredient in poultry feeding.

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