Serum Biochemistry, Organ Weight, Carcass Characteristics, Organoleptic Properties and Villi Morphometry of Nera Black Cocks fed Varying Levels of *Moringa oleifera* Leaf Meal

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Abstract
A total number of sixty-four (28 weeks old) matured Nera black cocks were randomly allotted to 4 dietary groups. Diet T1 (control) had no *Moringa Oleifera* leaf meal (MOLM) inclusion while diets T2, T3 and T4 contained graded levels of MOLM at 10%, 20% and 30% replacement for soya bean meal (w/w) respectively in a completely randomized design. All the serum biochemistry parameters evaluated differs significantly (P≤0.05) except albumin and cholesterol (P>0.05) among the dietary treatments. The weight of the kidney, heart, pancreas, proventriculus and spleen were influenced by the dietary treatments (P≤0.05). The weights of breast and empty gizzard increased (P≤0.05) linearly with MOLM inclusion while the wings, thigh and drum stick compared (P≤0.05) with those fed the control diet. The villi length and muscle thickness were significantly (P≤0.05) influenced by MOLM inclusion. The MOLM supplemented birds had longer villi than birds in the control group. The result of the this study showed that replacement of soybean meal with MOLM up to 30% inclusion in the diets of Nera black cocks was not detrimental to organ weights, carcass characteristics, sensory attributes and villi morphometrics. However, some serum parameters were adversely affected.

Keywords: carcass, cocks, moringa, serum parameters, sensory properties.

1. Introduction

The rapid growth of some tropical legume browse plants can be harnessed as a possible source of cheap protein for livestock feeding. Leaf meals do not only provide protein source but also some essential vitamins, minerals and oxycarotenoids [1]. Saewatt, et al. [2] have demonstrated that *Moringa oleifera* leaf meal provides natural antimicrobial/ medicinal properties, vitamins, minerals and proteins. Moringa leaf is also rich in carotene, ascorbic acid, iron and in the two amino acids that are generally deficient in other feeds, i.e. methionine and cystine [3]. Chemical analysis of *Moringa oleifera* on dry matter basis as reported by Yameogo et al. [4] indicated that the leaves contained 27.2% protein, 5.9% moisture, 17.1% fat and 38.6% carbohydrate while Bennett et al., [5] reported a crude protein, crude lipids and ash values of 26.4%, 6.5% and 12%, respectively for unextracted leaves. The variation in the reported values may be due to differences in agro-climatic conditions. According to Enechi and Odunwodu [6], *Moringa oleifera* leaves were reported to contain tannins (0.25), phytates (0.13), trysin inhibitor (0.04), saponins (0.05), oxalates

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(0.01) and low levels of cyanide (0.01). The levels of these anti-nutrients are low.

Feed is known to influence livestock performance. For instance, blood act as pathological reflector of the status of exposed animals to toxicant and other conditions [7]. As reported by Isaac *et al*., [8] animals with good blood composition are likely to show good performance. Laboratory test on the blood are vital tools that help detect any deviation from normal in the vital organs in animal or human body [9]. Feed has also been reported to influence meat quality. Meat quality attributes is both for the consumer's selection of fresh meat at the retail level and for the consumer's final evaluation and acceptance of a meat product at time of consumption [10-12].

In poultry production, researchers have reported on the effect of leaf meal on the growth performance of layer chicks [13], productive performance of laying hens [14-15], and broiler performance [16-17]. There is little information on blood characteristics, organ weight, carcass characteristics, organoleptic properties and villi morphometry of nera black cocks fed varying levels of *Moringa oleifera* leaf meal.

2. Materials and methods

This study was approved based on the ''principles of laboratory animal care (nih publication no. 85-23. Revised 1985''). This experiment was examined and approved by the appropriate ethics committee" (Ref./ LAU/FAS/IACUC/ANB-100599: 31st Mar., 2016). The experiment was conducted at the Poultry Unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria. Ogbomoso lies on longitude 4^0 15^1 East of the Greenwich Meridian and Latitude 8^0 07^1 North of the equator. It is about 145km North – Eastward from Ibadan, the capital of Oyo State. The altitude is between 300 and 600m above sea level. The mean annual temperature is about 27^0c while that of rainfall is 1247mm [18].

Fresh *Moringa oleifera* leaves were procured from the Teaching and Research Farm of Ladoke Akintola University of Technology, Ogbomoso. The leaves were processed into meal by air-drying the defoliated leaves to a constant weight before milling and there after incorporated into the test diet. Four diets were formulated with soya bean meal (SBM) as the main source of plant protein. The control diet (T1) contained 0% MOLM inclusion while diets T2, T3 and T4 were substituted with varying levels of MOLM at 10%, 20% and 30% replacement (w/w) respectively for SBM (Table 1).

A total number of sixty-four Nera black cocks at 28 weeks of age were used for the experiment. The cocks were randomly assigned into 4 dietary groups of 4 replicates with eight birds each in a completely randomized design. Experimental birds were procured from a reputable hatchery in Ibadan, Nigeria. All birds were fed *ad-libitum*.

Prior to commencement of experiment, birds were properly vaccinated against Newcastle disease, gumboro (IBD) and fowl pox disease. Wood shaving was used as litter material. Feed and clean water was supplied *ad-libitum* while proper cleaning and hygiene was maintained during the course of the experiment. At the end of the experiment, all the birds were starved overnight and two birds were randomly picked per replicate, tagged, weighed and slaughtered.

5ml of blood was collected sterilized glass bottles/tubes for each bird and were accurately labeled. Serum was obtained by centrifugation and serum samples were stored in a deep freezer (at minus 10^oC) until required for analysis. Blood ccholesterol were determined by spectrophotometric methods. Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Alanine phosphatase (ALP) was determined manually by spectrophotometric method as described by [19]. Total serum protein was determined using the biuret method as described by [20] while albumin was determined using the BCG (Bromocresol green] method as described by [21].

After slaughtering, incision was carefully made around the abdomen with a pen knife to create space through which the visceral were removed. The weight of the kidney, heart, liver, lungs, gizzard, proventriculus and pancreas were taken. The organs were weighed using the electronic sensitive weighing scale and their respective weights were recorded and expressed as a percentage of fasted live weight. The dressing percentage was calculated as follows: Dressing percentage=Carcass weight/Live weight×100 [22].

Samples of the jejunum were transversely cut, fixed in labelled bottle containing 10% formalin solution and taken to the laboratory for

histological analysis. Slides for microscopic examination were prepared using a thin layer cuts of the preserved organs, stained with eosin and haematoxylin as described by [23].

The histological examinations were done under light microscope (Olympus, Tokyo, Japan). Photomicrographs of the organ were taken with Authtek Camera (Leitz GmBh, Germany).

Table 1. Composition of experimental diet

Ingredients (%)	T1 (0%)	T2 (10%)	T3 (20%)	T4 (30%)
Maize	50.00	50.00	50.00	50.00
Corn Bran	5.00	5.00	5.00	5.00
Wheat offal	9.00	9.00	9.00	9.00
Soya bean meal	20.00	18.00	16.00	14.00
Moringa oleifera leaf meal	0.00	2.00	4.00	6.00
Fish meal	2.00	2.00	2.00	2.00
Palm kernel meal	5.00	5.00	5.00	5.00
Oyster shell	4.50	4.50	4.50	4.50
Bone meal	4.00	4.00	4.00	4.00
Methionine	0.05	0.05	0.05	0.05
Lysine	0.05	0.05	0.05	0.05
Breeder premix	0.20	0.20	0.20	0.20
Salt	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Crude protein (%)	17.80	17.50	17.30	17.00
Crude fiber (%)	7.30	7.30	7.40	7.40
ME(Kcal/Kg)	2,620	2,593	2,568	2,540
ME: Metabolizable energy				

Sensory evaluation was carried out on color, flavor, tenderness, juiciness and overall acceptability of breast samples from each slaughtered birds per replicate on a nine point hedonic scale using a 10 member panel [24]. The score was arranged in a descending order: the maximum score was given to extremely like condition while the lowest score was 1 for the poorest condition. All data collected were subjected to analysis of variance in a completely randomized design using SAS [25] software package and means were separated using Duncan multiple range test of the same package.

3. Results and discussion

Results: Table 2 shows the result of serum biochemistry of the matured black nera cocks fed varying levels of *Moringa oleifera* leaf meal. It was observed that all the serum biochemistry parameters differs significantly ($P<0.05$) except albumin and cholesterol which had no significant difference ($P>0.05$) among the dietary treatment. Linear increase in the level of MOLM resulted a decrease in the values of total protein such that a bird fed the control diet was significantly different from those fed T3 and T4. Conversely, increasing the levels of MOLM caused elevated response ($P<0.05$) in ALP, creatinine, TAG, ALT and AST when compared with birds on the control diet. Birds fed the control diet had the least ALP, creatinine, triglyceride and AST while the highest was recorded in birds fed T4. Nevertheless, birds on the control diet had the highest values for ADL and ACP, while birds on T3 had the lowest values. Although a quadratic response was observed as shown in Table 3. The weight of the kidney, heart, pancreas, proventriculus and spleen were influenced by the dietary treatments ($p<0.05$) except for the liver and lungs. Linear increases were observed in the parameters up to 20% replacement level of MOLM. Birds fed control and T3 were not significantly different ($p<0.05$) in the weight of kidney and heart but were significantly influenced ($p<0.05$) when compared with other dietary treatments. Linear increase in levels of MOLM resulted in the increased size of the pancreas ($p<0.05$). The quadratic response in the weight of proventriculus showed that the birds fed the control recorded the least weight ($p<0.05$). The weight of spleen was highest in birds fed T3 and was significantly different ($p<0.05$) from those fed other dietary treatments.

Carcass characteristic of breeder cocks fed *Moringa oleifera* leaf meal is shown in Table 4. The live weight, carcass weight, empty gizzard, drumstick, thigh, wings, breast, neckand back were significantly influenced ($p<0.05$) by the dietary treatments while the bleed weight and shank was not influenced ($p>0.05$) by the dietary treatments. There was a linear increase in the size of the breast but a linear decrease in the size of the neck as the inclusion level increases while other significant parameters had quadratic changes in value.

The analysis of the panelist response showed that they were indifferent ($p>0.05$) to the samples provided as shown in Table 5.

Table 2. Serum biochemistry of breeder cocks fed *Moringa oleifera* leaf meal diet

Parameters unit	T1 (0%)	T2 (10%)	T3 (20%)	T4 (30%)	SEM
Total protein (g/dL)	5.17^{a}	4.40^{ab}	3.40^{c}	3.95^{bc}	0.22
Albumin (g/dL)	1.71	1.61	1.58	1.54	0.03
Alkaline phosphate (UI/L)	44.16^{c}	59.34^{b}	70.38^{a}	76.82^{a}	3.84
Creatinine (mg/dL)	17.85^{c}	24.55^{b}	22.32^{ab}	31.24^{a}	1.60
Cholesterol (mg/dL)	120.83	133.33	150.00	133.33	8.92
Triglyceride (UI/L)	40.22^{b}	53.26^{ab}	51.09^{ab}	58.70^{a}	2.98
Alkaline phosphate (UI/L)	23.28^{b}	38.00^{ab}	45.92^{a}	44.42^{a}	3.37
Aspartate Aminotransferase	52.37^{b}	92.89^{ab}	91.00^{ab}	129.21^{a}	9.82
High density lipoprotein (mg/dL)	49.45^{a}	29.67^{ab}	19.78^{b}	34.07^{ab}	4.37
Acid phosphate (UI/L)	5.36^{a}	4.13^{ab}	1.87^{b}	3.75^{ab}	0.57

^{ab}means with different subscript along the row are significantly different ($p<0.05$)

Table 3. Organ weight (% of live weight) breeder cocks fed *Moringa oleifera* leaf meal

Parameters (%)	T1 (0%)	T2 (10%)	T3 (20%)	T4 (30%)	SEM
Liver	1.11	1.21	1.26	1.21	0.04
Kidney	0.32^{a}	0.19^{b}	0.30^{a}	0.22^{b}	0.02
Lungs	0.38	0.37	0.42	0.39	0.01
Heart	0.63^{a}	0.56^{ab}	0.64^{a}	0.49^{b}	0.02
Pancreases	0.13^{b}	0.15^{ab}	0.17^{a}	0.17^{a}	0.01
Provent	0.19^{c}	0.28^{a}	0.30^{a}	0.23^{b}	0.01
Spleen	0.12^{b}	0.10^{b}	0.18^{a}	0.13^{b}	0.01

^{ab}means with different subscript along the row are significantly different ($p<0.05$)

The color score ranges from 5.80 (T1)–6.70 (T3) while the flavor recorded 3.40, 3.20, 4.20 and 4.30 in birds fed T1-T4 respectively unlike 4.10, 5.20, 5.00 and 4.90 for birds fed T1-T4 respectively for tenderness. Birds fed T4 had the highest score for juiciness while those fed T3 had the least.

Table 4. Carcass characteristic of breeder cocks fed *Moringa oleifera* leaf meal based diets

Parameters (%	T1 (0%)	T2 (10%)	T3 (20%)	T4 (30%)	SEM
Live weight (g/b)	2700.00^{ab}	2875.00^{a}	2650.00^{ab}	2525.00^{b}	46.62
Bleed weight	91.48	91.12	92.97	92.78	0.43
Carcass weight	69.44^{b}	86.05^{a}	68.49^{b}	70.29^{b}	2.83
Empty gizzard	1.20^{b}	1.21^{b}	1.42^{a}	1.33^{a}	0.03
Drum	11.99^{a}	12.23^{a}	7.87^{b}	11.41^{ab}	0.71
Thigh	13.06^{a}	13.14^{a}	11.99^{b}	13.16^{a}	0.16
Wing	8.41^{b}	8.88^{ab}	8.78^{ab}	9.25^{a}	0.12
Breast	12.85^{b}	13.65^{ab}	13.97^{ab}	14.40^{a}	0.22
Neck(%)	8.52^{a}	7.98^{ab}	7.73^{ab}	7.22^{b}	0.20
Back(%)	12.87^{ab}	11.86^{b}	13.09^{a}	12.69^{ab}	0.20
Shank(%)	3.93	3.99	3.88	3.87	0.02

^{ab}means with different subscript along the row are significantly different ($p<0.05$)

The lowest score for texture was recorded in birds fed T1 (4.70) while those fed T4 (6.30) had the highest. The overall acceptability score was 6.80, 5.90, 6.60 and 6.70 for birds fed T1-T4 respectively.

Table 5. Organoleptic properties of breeder cocks cocks fed varying levels of *Moringa oleifera* leaf meal

Parameters	T1 (0%)	T2 (10%)	T3 (20%)	T4 (30%)	SEM
Color	5.80	6.50	6.70	6.30	0.22
Flavor	3.40	3.20	4.20	4.30	0.31
Tenderness	4.10	5.20	5.00	4.90	0.22
Juiciness	5.90	5.60	5.50	6.40	0.22
Texture	4.70	4.90	5.40	6.30	0.27
Overall acceptability	6.80	5.90	6.60	6.70	0.21

The villi morphometry of Nera black breeder cocks fed varying levels of *Moringa oleifera* leaf meal is shown in Table 6. The Villi height and Muscle thickness were significantly ($P<0.05$) influenced by the dietary treatment while the villi width and Cryptal depth were not significantly different ($P>0.05$) among the dietary treatment. Birds fed MOLM diets had a higher villi height than those fed the control diet, unlike T2 which recorded the highest value for muscle thickness while birds fed T4 had the least though not significantly different ($P>0.05$) from those fed the control diet.

Discussion: Unlike the result of this study, serum metabolites including total protein, albumin, globulin, cholesterol, Alkaline phosphate (ALP), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were not significantly influenced when Amao et al. [26] fed cocks with cocoabean shell supplemented with Vitamin E and B-glucan. The authors further explained that the observed similarities in the values of ALP, ALT and AST indicated that there was no tissue damage caused by the diets.

Table 6. Intestinal (Villi) parameters of breeder cocks fed varying levels of *Moringa oleifera* leaf meal

Parameters (μm)	T1 (0%)	T2 (10%)	T3 (20%)	T4 (30%)	SEM
Villi height	841.73^{b}	1040.27^{ab}	904.40^{b}	1241.40^{a}	56.35
Villi width	105.43	109.30	108.70	103.52	3.64
Cryptal depth	205.80	304.50	298.53	300.87	22.55
Muscle thickness	65.13^{b}	87.43^{a}	75.27^{ab}	62.87^{b}	3.80

ab: means along the same row with different superscript are significantly different ($p<0.05$)

On the other hand, the higher serum total protein (TP) content of broilers receiving dietary MOLM might be an indication of the good protein content and/or quality of the leaf meal since serum protein indicates the protein retained in the animal body [27]. But the depressed TP at 20% MOLM inclusion level corroborates the findings of Tijani et al. [28] that at such level the adverse effect of Moringa phytotoxins such as lecithin, moringin, moringinine, glucosinolates, tannins, nitrite, oxalate and phytate [29] was pronounced. It has been reported by [30] that elevated serum enzyme activities such as AST, ALT and ALP is an indication of heart, kidney and/or liver damage due to cellular destruction caused by toxins.

Meanwhile, significant differences were not observed for ALT, ALP and ACP when Ojediran et al. [31] fed broilers with differently processed *Jatropha curcas* kernel meals.

The non-significant liver weights may indicate that the liver being the organ of detoxification was not overlabored to result in hypertrophy. Increase in the weight of pancreas and proventriculus in the birds fed MOLM indicates that those organs increased their secretory functions.

Okosun and Eguaoje [32] attributed such increase in weight especially for spleen could be due to the higher solubility and easy flow of feed in aqueous milieu of the gastro-intestinal tract.

Comparable live weight of the birds conform with the report of Ojediran et al. [33] when broiler chickens were fed low crude protein diets supplemented with varying levels of dietary lysine. Onunkwo et al. [34] earlier reported that MOLM influenced carcass weights (wings, drumstick, kidney, shank, breast, etc) of broiler chicken. High carcass yield observed in this study may be related to the live weight. Although, increased in carcass weight suggests more nutrient bioavailability for muscle development [35] because all the diets compared favorably with the control. Extra muscular activity of the gizzard may have been responsible for the increase in weight. This is unlike the report of Okosun, and Eguaoje (2017) [32] who fed cockerel graded levels of cassava grit supplemented with moringa leaf meal. Observations on the drumstick, thigh wings and back weights did not follow a definite pattern. This negates the findings of Ojediran et al. [33]. Significant linear increase recorded in the breast weight as the MOLM increases was not in tandem with the earlier report of Okosun, and Eguaoje [32]. However, the weight of neck decreased.

The non-significant sensory analysis shows that consumer preference was not adversely influenced unlike the result of Akinwumi et al. [36]. The result of this study corroborates the finding of Aderinola et al. [37], who reported that organoleptic properties of broilers fed MOLM based diets were not different. Consumers reject products in which the color departs from the normal appearance [38] and the meat color of diets fed MOLM appeared normal. The trained panelists observed that meat samples from cocks fed MOLM were moderately light in color unlike those fed the control which was slightly light. This is similar to the observation of Karthika et al.[39] who reported that color could be influenced by myoglobin content, composition, and physical state of muscle and meat structure. Although, the high Beta–carotene and vitamin C (6.26mg/100m/extract) content of the leaf according to Madukwe et al. [40] may have contributed. Flavour tends to develop with age while juiciness depends largely on the fat content of the carcasses [41]. Samples from birds fed the control and T2 were moderately perceptible while those fed T3 and T4 were slightly perceptible in flavor. Intramuscular lipid and moisture content/water holding capacity of meat is directly related to juiciness. In combination with water, melted lipid constitutes a broth which when retained in meat is released upon chewing [39]. Omojola [42] reported that sex had significant effect on juiciness and tenderness of duck meat. The observation in this study therefore may not be unconnected with the sex of the birds used in this study. Meanwhile, [36], revealed that bird type could influence consumer preference for juiciness, flavor and tenderness. Birds fed the control diets had a slightly tough meat unlike those on MOLM which were intermediate in tenderness. Samples from the birds fed the control diet were slightly dry while those fed MOLM diets were slightly juicy. In terms of the texture, birds on T1-T3 were intermediate in texture unlike those fed T4 which was slightly fine. Meat overall acceptability determines the quality. Overall rating for acceptability compared favorable among birds fed MOLM compared with those fed the control. Overall, T1 and T4 had moderate overall acceptability while those fed T2 and T3 had slight overall acceptability. This is agreed with Omojola et al. [42] that acceptability may not be influenced by sex of the bird. In addition, Abu et al. [43], recorded that the organoleptic values were influenced for juiciness, taste, color and overall acceptability for birds fed cassava peels and leaf meal as replacement for soya bean meal to 20% inclusion level.

No definite pattern was observed in the villi morphometrics. V illi height and muscle thickness were significantly influenced in this study but [33] observed that amino acid supplementation of low protein diets had significant effect on villi height, villi width, cryptal depth and muscle thickness of broiler chickens. Higher villi height could translate to greater surface area for absorption of nutrients while increased muscle thickness may signify that there was no erosion of the intestinal layers. Nkukwana et al. [44] concluded that the ability of birds to efficiently utilize a feed is measured by the response of the villous to the feed form.

4. Conclusions

It can be concluded that replacing soybean meal with up to 30% MOLM does not adversely affect the organ weight, carcass cuts, organoleptic

properties and villi morphology of Nera black cocks unlike some serum parameters.

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