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Original Research Article

Influence of *Moringa oleifera* leaf meal on egg lipids and blood constituents of laying hens

ABSTRACT

The influence of Moringa leaf meal on egg lipids and blood constituents of laying birds was examined using 120 ISA Brown layers that were 74 weeks old. The hens were assigned randomly to four groups namely, T_1, T_2 T_3 and T_4. The design of the experiment was the completely randomized design. Each treatment contained 30 birds which had 10 layers in each of the three replicates. The diet for each treatment contained 0, 0.5, 1.0 and 1.5 % of the moringa leaf meal in the layers mash which was composed of 2700Kcal ME /kg,15 % crude protein, 5 % fat, 10 % crude fibre, 3.5 % calcium and 0.4 % phosphorus. On completion of the study, 20 eggs were picked from each replicate within 72 hours for the evaluation of the cholesterol, triglyceride, high-density lipoprotein (HDL) and low-density lipoprotein (LDL). Samples of the blood were obtained from three hens per replicate for haemoglobin (Hb), red blood cell (RBC), packed cell volume (PCV), white blood cell (WBC), Mean corpuscular volume (MVC), Mean corpuscular hemoglobin(MCH) and Mean corpuscular hemoglobin concentration (MCHC) as well as the study of the alanine amino transferase (ALT), aspartate aminotransferase (AST), total protein, glucose, serum total cholesterol, triglyceride, HDL and LDL. The result revealed that the HDL of the eggs was influenced by the diets such that it was significantly ($P < 0.05$) greater in T_2 and T_3, of the fresh eggs and favoured the good cholesterol, HDL of the eggs after seven days (end of the first week) of storage. The Hb, PCV, MCV and MCH of the blood were highly ($P < 0.05$) influenced by the treatments while the other haematological and serum parameters studied were not affected. In conclusion, the leaf meal had favourable impact on the eggs lipids up to the seventh day (end of the first

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week) of storage and minimal effect on the blood constituents of the layers at the level of 0.5 – 1.5 % of the feed.

Keywords: Egg lipids, Haematology, Laying birds, Moringa meal, Serum

1. INTRODUCTION

The poultry industry has continuously intensified her commercial production to satisfy the need of the growing population in Nigeria. Thus, there is need to intensify the supply and quality of poultry feed to correspond with the demand and production needs. Unfortunately, in Nigeria, the available conventional poultry feeds, though expensive, are very poor in quality [1,2]. This poses an adverse effect on the production benchmark. The continuous poor quality of the feed could be traced to the competition of human and livestock for food materials such as maize, soya bean, wheat, groundnut, etc, the increasing cost of feedstuff due to the scarcity of conventional ingredients. Intensive and continuous efforts are being made by Animal Nutritionists to determine the nutritional content of agro-industrial by-products, shrubs and others ingredients to replace the costly ingredients in the feed for birds [3] or improve the feeds. Thus, researchers have recommended the inclusion of certain percentage of less economic raw materials including by-products of plants and animals in feed production [4]. In the bid to improve the quality of commercial poultry feed, researchers advocate the incorporation of the feed with nutritive additives (vitamins and minerals) and plant and animal materials that are of less economical value to improve the productivity of the animals. One of the materials which have been closely studied and used as feed additive is the leaves of *Moringa oleifera.*

Blood is a vital part of clinical pathology and diagnostic process used to ascertain the healthiness of the animals. According to [5], haematological parameters are useful indicators of the functional status of animals and its variations are therefore necessary in assessing the

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reactions of the animals to various biological conditions. Also, [6] ted that the haematological characteristics of livestock had been observed to be a feature used in assessing the reaction of animals to the diets while [7] ted that serum biochemical parameters may provide useful information for the assessment of the health condition of birds.

Although in recent times, there had been positive response to conventional poultry feed supplementation as a result of the call by Animal Nutritionists to fortify feeds with nutritive additives (vitamins and minerals) and/or plant and animal materials. Most of the research have been carried out on the incorporation of commercial feed with hormones, vitamins, ginger, agro-industrial by-products, animal by-products and leaves of plants.

Moringa oleifera, commonly called moringa in Nigeria, is one of the most studied plants with respect to human uses and animal feed additive due to it's enormous nutritive and medicinal properties [8]. According to [9], various parts of the plant, such as the leaves, roots, seeds, bark, fruits, flowers and immature pods act as cardiac and circulatory stimulants, possess antipyretic, antitumor, anti-inflammatory, antiulcer antispasmodic, antihypertensive, diuretic, cholesterol lowering, antidiabetic, antibacterial, antioxidant, hepatoprotective and antifungal activities being used for treating various ailments in the traditional system of medical practice, particularly in South Asia. The proximate composition of dried moringa leaf as given by [10] dry matter 94.503mg/g; fatty acid 1.636mg/g; carbohydrate 54.183mg/g; energy 1416.9mg/g; protein 22.523mg/g; fat 1.473mg/g; fibre 9.003mg/g; ash 7.000mg/g and moisture 5.487mg/g. However, the studies on how to use of moringa leaf as feed additive are mainly due to its high nutritional content. Therefore, it will be important to study if the egg lipids and blood constituents of layers are influenced by the inclusion of the leaf in the diets.

2. MATERIALS AND METHODS

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2.1 Experiment location

The study was carried out in the Poultry Section of the Research and Teaching Farm of the University of Port-Harcourt, Choba, Rivers State. Rivers State is located on the 4° 45'N, 6° 50'E (4.750° N, 6.833° E), having an annual average temperature of 26° C (78.8° F).

2.2 Experimental animals and design

ISA Brown strain of layer chicken numbering 120 were collected from the layers' Section of the Research and Teaching Farm were used for the study. The hens were randomly shared to four treatment groups, having 30 birds each. The treatment group denoted T_1, served as the control, while the other three were designated as T_2, T_3, and T_4. Each treatment was further shared into 3 replicates, containing 10 birds each.

2.3 Preparation of the diets and experimental procedure

Fresh Moringa leaves were harvested from the trees in Port-Harcourt metropolis. The leaves were sun-dried to reduce the moisture content and milled. They were stored in an air-tight container until they were used. Commercial layers' mash containing metabolizable energy of 2700Kcal/kg, crude protein 15.0%, fat 5.0%, crude fibre 10.0%, calcium 3.5% and phosphorus 0.4%, as indicated on the bag label was bought from a reliable livestock feed company. The T_1 (control diet) T_2, T_3, and T_4 (experimental diets) were prepared by incorporating the prepared grind moringa leaf at 0g (0%), 5g (0.5%), 10g (1.0%) and 15g (1.5%) per kg feed respectively into the mash and mixed thoroughly. The hens were weighed and randomly assigned to the battery cages according to the treatments and were fed for 12weeks. All the groups had the same environmental condition and management.

2.4 Data collection

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At the completion of the 12 weeks experiment, 20 eggs were picked per replicate and analyzed for cholesterol, triglyceride, high-density lipoprotein (HDL) and low-density lipoprotein (LDL). Five of the eggs were analyzed on the day they were laid while five were analyzed weekly for three weeks from each replicate. Three blood samples were collected from three birds per replicate for laboratory analyses. Samples of blood were collected through the cutaneous ulnar vein of the wing. Bottles treated with ethylene diamine tetra-acetate (EDTA) which prevented the formation of blood clots was used collecting the blood samples for hematological analysis while plain/untreated bottles were used collecting the samples for serum biochemical analysis. The blood contained in the EDTA bottles were analyzed for haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV). Data obtained from the haematological analysis were used to compute the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). The serum obtained was used to determine the aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total protein, blood cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), and glucose levels.

2.5 Statistical analysis

The data generated were subjected to the one-way analysis of variance (ANOVA) using the SPSS Inc. package. The means of the treatment were compared using Duncan's procedure to examine whether there were notable differences in the egg lipid and blood constituents.

3. RESULTS

Table 1 shows the result of egg lipid of the hens fed the varying levels of moringa leaf meal. The egg lipid profile revealed that the good cholesterol, high-density lipoprotein (HDL) was significantly ($P < 0.05$) higher in T_1 and least in T_4. The levels of the HDL in T_2 and T_3 did

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not differ from the T_1 (control) and T_4. There was similarity in the values of cholesterol, triglyceride and low density-lipoprotein (LDL).

Table 1: Influence of treatments on egg lipid of the laying birds

	T_1 (0%)	T_2 (0.5%)	T_3 (1.0%)	T_4 (1.5%)	SEM
TC (mmol/l)	25.00	24.33	24.33	23.66	0.22
TGL (mmol/l)	6.20	6.20	6016	6.20	0.02
HDL (mmol/l)	9.30^{a}	6.53^{ab}	5.83^{ab}	3.46^{b}	0.77
LDL (mmol/l)	0.16	0.10	0.13	0.13	0.10

[ab] Means within rows with no common superscript are significantly different ($P < 0.05$)
TC = Total cholesterol, TGL= Triglyceride, HDL= High-density lipoprotein, LDL= Low-density lipoprotein, SEM= standard error of mean

The result from the stored eggs revealed that the good cholesterol, HDL was better at the end of the first week in eggs obtained from T_3 and T_4, although not different from T_2 with a correspondingly lower level of the LDL. The total cholesterol and triglyceride were not altered at the end of the first week of storage. At the end of the second week of storage, the cholesterol was highest in T_4 and lower in T_1, T_2 and T_3 while the HDL was best in T_1 and worst in T_2 with a correspondingly lower value of LDL in the control and worst (highest value) in T_2. The total cholesterol after week three was higher in T_1, though not different from T_2 and T_3 and lower in T_4 while the triglyceride was highest in T4, although not different from T_3 and lower in T_1 and T_2. The HDL and LDL did not differ across the treatments after the third week.

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Table 2: Influence of storage on the egg lipids

Parameter (mmol/L)	**T_1 (0%)**	**T_2 (0.5%)**	**T_3 (1.0%)**	**T_4 (1.5%)**	**SEM**
Week 1					
Total Cholesterol	24.00	25.50	25.00	23.50	0.50
Triglyceride	6.05	6.15	6.05	6.10	0.29
HDL	2.15^{b}	3.50^{ab}	4.75^{a}	5.30^{a}	0.51
LDL	8.15^{a}	3.05^{b}	1.00^{b}	1.00^{b}	1.19
Week 2					
Total Cholesterol	21.50^{b}	20.50^{b}	19.50^{b}	23.50^{a}	0.59
Triglyceride	7.05	6.80	6.70	7.00	0.14
HDL	3.75^{a}	1.30^{c}	1.80^{bc}	2.10^{b}	0.35
LDL	1.00^{c}	8.50^{a}	4.40^{b}	5.55^{b}	1.20
Week 3					
Total Cholesterol	26.00^{a}	23.50^{ab}	25.00^{ab}	20.50^{b}	0.90
Triglyceride	6.35^{b}	6.05^{b}	6.50^{ab}	7.00^{a}	0.14
HDL	1.85	1.85	1.70	1.70	0.10
LDL	11.30	9.50	10.90	5.35	1.32

^{ab} - Means within rows with different superscript are significantly different ($P < 0.05$)
HDL – High-density lipoprotein, LDL – Low-density lipoprotein

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Table 3 shows the results of the haematological parameters of the layers that were fed the various percentages of the moringa leaf meal. There were sharp ($P < 0.05$) differences amongst the treatment groups in the haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV), while no sharp ($P > 0.05$) differences were noticed in red blood cells (RBC), white blood cells (WBC), and mean corpuscular haemoglobin levels (MCHC). In all the blood parameters that were affected sharply, the concentrations of T_1 and T_3 were higher but similar to each other while the T_2 and T_4 were significantly lower.

Only T_1 had the normal range of Hb for chicken as given by [11], while in the other treatment groups: T_2, T_3, and T_4, Hb was sharply ($P < 0.05$) less than the normal range. The hens were however, physically healthy as no mortality was recorded but the possibility of anaemia could not be completely ruled out. Similarly, PCV, MCH and MCV of the treatments were found to be lower than normal.

Table 3: Influence of treatments on the haematological parameters of laying birds

Parameters	**T_1 (0%)**	**T_2 (0.5%)**	**T_3 (1.0%)**	**T_4 (1.5%)**	**SEM**
Hb (g/dl)	7.00^{a}	5.00^{b}	6.46^{ab}	4.76^{b}	0.36
PCV (%)	21.00^{a}	15.00^{b}	19.33^{ab}	14.33^{b}	1.07
RBC $\times 10^{12}$/l	3.46	2.76	3.33	2.73	0.13
MCH (pg)	20.21^{a}	17.97^{b}	19.35^{ab}	17.39^{b}	0.42
MCV (fl)	60.65^{a}	53.93^{b}	57.88^{ab}	52.30^{b}	1.25

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MCHC (%)	33.33	33.32	33.43	33.26	0.04
WBC $\times 10^{12}$/l	3.63	3.73	4.20	3.66	0.14

[ab] Means within rows with no common superscript are significantly different ($P < 0.05$)
Hb = Haemoglobin, PCV = Packed cell volume, RBC = Red blood cell, MCH = Mean corpuscular haemoglobin, MCV = Mean corpuscular volume, MCHC = Mean corpuscular haemoglobin concentration, WBC = White blood cell, SEM = standard error mean

From the results of the serum biochemistry of layers fed the varying percentages of the leaf meal in Table 4, it was revealed that the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose and high-density lipoprotein (HDL) were not significantly ($P > 0.05$) different but those of total protein, cholesterol, triglyceride and low-density lipoprotein were significantly ($P < 0.05$) different. The total protein, cholesterol and triglyceride was increased in T_2 but decreased in T_3. Among the serum parameters, only the glucose and cholesterol were within the normal range of 6.9 - 11.1 and 1.3 - 3.8 respectively while the other parameters had just one or two of their values tallying with the normal value.

Table 4: Influence of treatments on the serum biochemistry of laying birds

	T_1 (0%)	**T_2 (0.5%)**	**T_3 (1.0%)**	**T_4 (1.5%)**	**SEM**
AST (u/l)	83.66	87.00	75.00	89.00	4.77
ALT (u/l)	11.66	13.33	8.00	7.33	1.37
TP (g/l)	48.00[ab]	54.33[a]	45.66[b]	50.66[ab]	1.31
GLU (mmol/L)	8.70	10.20	9.73	8.90	0.51
TC (mmol/L)	2.26[bc]	3.46[a]	1.43[c]	2.93[ab]	0.27

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TGL (mmol/L)	2.76[b]	5.06[a]	2.43[b]	4.40[ab]	0.41
HDL (mmol/L)	0.86	0.60	0.73	0.13	0.12
LDL (mmol/L)	0.13[ab]	0.10[b]	0.16[ab]	0.50[a]	0.06

[abc] Means within rows with different superscript are significantly different ($P < 0.05$)
AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, TP = Total protein, GLU = Glucose, TC = Total cholesterol, TGL = Triglyceride, HDL = High-density lipoprotein, LDL = Low-density lipoprotein, SEM= standard error mean

4. DISCUSSION

The reduction in HDL as the percentage of the leaf in the diet increased and the absence of differences in cholesterol, triglyceride and LDL of the eggs tallied with the report of [12] who stated that lower levels of inclusion of the leaf meal of moringa in the diet of laying hens reduced the lipid content of the eggs, improved the egg quality without affecting the feed consumed, feed conversion ratio, percentage of egg lay and egg mass. It was however contrary to the finding of [13] who reported an elevation in the HDL, and reduction in LDL and cholesterol in the eggs of layers fed with moringa leaf meal. According to [14] HDL helps to transport cholesterol to the liver for its excretion. It was established by [15,16] that increased levels of HDL lead to lesser danger of heart disease, stroke and health problems This implied that the consumption of eggs from the hen that were fed the highest level of the leaf meal will lead to low intake of dietary HDL since the concentration of HDL was greatly reduced. The absence of differences in the cholesterol, triglyceride and LDL indicated that the varying percentages of moringa leaf meal did not affect the parameters. This was contrary to the report of [17] who found decreasing values of cholesterol as the duration of storage increased for eggs that were obtained from birds fed different commercial layers feed.

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The higher level of HDL in the eggs and the corresponding decrease in LDL in T_3 and T_4 which was similar to T_2 at the end of the 7th day (first week) of storage implied that storing eggs for a week from hens fed the 0.5, 1.0 and 1.5 % moringa leaf could be beneficial for human consumption. Increased levels of HDL had been reported to reduce the risk of heart disease, stroke and health problems [15,16]. However, the lower levels of the HDL in T_2 – T_4 at the end of the 14th day (second week) of storage with the corresponding higher level of LDL in T2 [illegible]l not be good health. Similar decreased levels of HDL at the end of the first two weeks of egg storage (in eggs collected from normal feeding, T_1 and four hours restricted feeding, T_2) with a corresponding high LDL was reported by [18] [illegible]he higher cholesterol content of the eggs from T_4 at the end of day 14 (second week) of storage tallied with the report of [18] who reported increased levels of egg cholesterol after two weeks of storage in eggs obtained from hens fed normally (T_1), four hours restricted feeding (T_2) and eight hours restricted feeding (T_3). The absence of variations in the HDL and LDL coupled with the decreasing trend of the cholesterol and increasing trend of the triglycerides after the third week of storage could be as a result of the effect of the treatments.

The reducing levels of the Hb, PCV, MCH and MCV as the percentage of moringa meal in the feed increased supported the finding of [19] [illegible]o found a decrease in the haematological parameters when moringa leaf meal was provided for broiler chickens. The RBC, MCHC and WBC which had no differences and maintained the normal values indicated that the addition of moringa leaf in the feed of the hens had no interaction with the production and physiology of the RBC and WBC. It, therefore, implied that the hens were not anaemic since no difference existed in the MCHC. According to [20] [illegible]en differences are not observed in hematologic values, yet they are within their normal range implies that no infection, stress or inflammatory situation may be present. The PCV usually gives good information especially

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when the animal has not shown any signs of illness [21]. The decreased PCV may have affected the calculated MCH and MCV values.

The concentrations of the aminotransferases, AST and ALT which were similar was an indication that the enzymes' biochemistry were unaffected and the livers of the hens were not damaged by the various levels of the leaf meal in their diets. The result obtained for AST were not different from [22] while the ALT tallied with those of [23]. The variation observed in the total protein level tallied with the report of [23] who reported difference in total protein of the blood in broilers that were fed the same leaf meal. According to [24] the difference could be linked to the time of lay which causes considerable increase in plasma total protein prior to egg laying due to an estrogen-induced elevation of the globulins. The glucose, cholesterol and triglyceride which had normal values implied that there was normal metabolic activity in the system of the birds. This implied that the endocrine gland, pancreas and liver of the hens were working normally in the birds since higher or lower concentrations of glucose, cholesterol and triglyceride is an indicator of disorders and malfunctioning of the liver and pancreas which can lead to lipemia and fatty liver degeneration among others. The increased triglyceride in T_2 and T_4 tallied with the report of [25] who found higher levels of triglycerides in the blood of layers when moringa leaf meal was used in their feed and attributed it to estrogen synthesis from the steroids in the leaves. This finding was at variance with the report of [19] who studied the effect of moringa in broiler birds. This finding was in line with [26] who reported differences (P <0.05) in the triglycerides of birds fed with different percentages of moringa leaf meal. The uniform level of the serum HDL showed the minimal influence of moringa leaf on the birds.

The bad cholesterol, LDL which was highest in T_4 and similar to T_1 and T_3 while it was least in T2 implied that no link existed between the serum concentration of the LDL and the LDL of the fresh eggs.

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5. CONCLUSION

This present study indicated that the inclusion of the leaf meal of moringa in layers' diet favoured the good cholesterol, HDL of the fresh eggs at levels of 0.5 and 1.0 % inclusion and the stored eggs at the end of day 7 (first week). However, an indication of possible negative effect of the inclusion of moringa leaf in the diets of layers was shown in the haematological parameters which decreased sharply in Hb, PCV, MCH and MCV (although values obtained in T_3 with the 1.0 % moringa inclusion was higher like the control) indicating the presence of anaemia, although the hens were physically healthy. Thus, eggs that are collected from hens fed moringa leaf meal at levels of 0.5 – 1.5 % should be consumed within one week of storage.

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