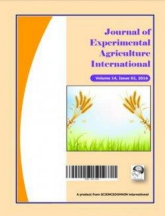

Journal of Experimental Agriculture International

22(2): 1-9, 2018; Article no.JEAI.40432
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Influence of *Moringa oleifera* Leaf Meal on Egg Lipids and Blood Constituents of Laying Hens

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Authors' contributions

This study was conducted by the both authors. Authors LAFA and NO together designed the study, collection of data and carried out the statistical analysis. Both authors jointly read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2018/40432
Editor(s):
(1) Rusu Teodor, Professor, Department of Technical and Soil Sciences, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania.
Reviewers:
(1) Anand Kumar Pathak, Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu, India.
(2) Bilgehan Yilmaz Dikmen, University of Uludağ, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/24050

Original Research Article

Received 27th January 2018
Accepted 4th April 2018
Published 7th April 2018

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 2700 Kcal ME /kg,15% crude protein, 5% fat, 10% crude fibre, 3.5% calcium and 0.4% phosphorus. At the end of the experiment, 20 eggs of similar weight 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 aminotransferase (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

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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 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 according to Carew [1] and Akinola and Ekine [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 and 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 according to Bolu [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 economic value to improve the productivity of the animals. *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 its enormous nutritive and medicinal properties [5]. According to Anwar et al. [6] the 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. Thus, *Moringa oleifera* leaf is one of the materials which have been closely studied and used as feed additive in poultry production. Bamishaiye et al. [7] found that the presence of some phytochemicals like tannins, saponins, phenols, alkaloids, phlobatannins and flavonoids in moringa leaves led to its medicinal action when used as a therapy. Ferreira et al. [8] reported that moringa leaf was a good indigenous source of highly digestible protein, methionine and cysteine (essential sulfur-containing amino acid), potassium, calcium, iron, phosphorus, magnesium, vitamins and phenolic compounds. According to Lala et al. [9] inclusion of moringa leaf in diet for laying hens led to reduced egg cholesterol and low density lipoprotein (LDL).

Blood is a vital part of clinical pathology and diagnostic process used to ascertain the healthiness of the animals. According to Khan and Zafar [10] haematological parameters are useful indicators of the functional status of animals and its variations are therefore necessary for assessing the reactions of the animals to various biological conditions. Also, Madubuike and Ekenyem [11] stated that the haematological characteristics of livestock had been observed to be a feature used in assessing the reaction of animals to the diets while Kudair and Al-Hussary [12] stated that serum biochemical parameters may provide useful information for the assessment of the health condition of birds.

The proximate composition of dried moringa leaf as given by Adeyemi et al. [13] is dry matter 94.503 mg/g; fatty acid 1.636 mg/g; carbohydrate 54.183 mg/g; energy 1416.9 mg/g; protein 22.523 mg/g; fat 1.473 mg/g; fibre 9.003 mg/g; ash 7.000 mg/g and moisture 5.487 mg/g. Since the studies on how to use moringa leaf as feed additive are mainly due to its high nutritional content and the deposition of cholesterol in egg yolk is affected by nutrition according to Hargis et al. [14] it will be important to learn how egg lipids and blood constituents of layers are influenced by the inclusion of the leaf of moringa in the diets.

2. MATERIALS AND METHODS

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, Nigeria. Rivers State is located on the 4º45'N, 6º50'E (4.750ºN, 6.833ºE), having an annual average temperature of 26ºC (78.8ºF).

2.2 Experimental Animals and Design

In the study, a total of 120 ISA Brown strain egg layers were collected from the layers' Section of the Research and Teaching Farm and 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 for one week to reduce the moisture content and milled. They were stored in an air-tight container until they were used. Commercial layers' mash which according to the manufacturer contained metabolizable energy of 2700 Kcal/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 0 g (0%), 5 g (0.5%), 10 g (1.0%) and 15 g (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 (morning and evening) for 12 weeks. All the groups had the same environmental condition and management.

2.4 Data Collection

At the completion of the 12 weeks' experiment in July, 20 eggs of similar weight were picked (in the morning and evening) within 72 hours and stored in a room (in the month of July) where the average temperature of the day was 28.7ºC and at night 22.3ºC while the humidity was about 86%. The eggs were analyzed for cholesterol, triglyceride, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in the morning of the due dates. Five of the eggs were analyzed on the day they were laid while five were analyzed weekly for three weeks from each replicate. The eggs were weighed, then, hard boiled and allowed to cool. Thereafter, they were carefully cracked and the yolk was separated from the albumen. One gramme of each of the yolk was extracted and the samples homogenized and filtered. The procedure of analyzing cholesterol using the Randox test kit UK was followed, adding $CaCl_2$ to the filtrate to aid the extraction of the aqueous phase of the non-lipid materials. Randox reagent was added to the extracted samples and the extracted lipids were incubated in a laboratory oven at 37ºC for 5 minutes and the absorbance of sample done using spectrophotometer. From the result obtained, the total cholesterol, HDL, LDL and total triglycerides were calculated.

A total of 24 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 (12 samples from the four treatments) while plain/untreated bottles were used for collecting the samples for serum biochemical analysis (12 samples for the four treatments). 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.

Haemoglobin (Hb) was determined by the cyanmethemoglobin method by converting haemoglobin to cyanmethemoglobin through the addition of potassium cyanide and ferricyanide with absorbance of 540 nm in a photoelectric calorimeter against a standard solution as described by Bhaskaram et al. [15]. The packed cell volume (PCV) was obtained using the haematocrit method described by to Dacie and Lewis [16] while the method of Jain [17] was

used to determine the red blood cell (RBC). The Hb, PCV and RBC were used to obtain the mean corpuscular hemoglobin (MCH = Hb ÷ RBC x 10), mean corpuscular volume (MCV = PCV ÷ RBC x 10), and mean corpuscular hemoglobin concentration (MCHC = Hb ÷ PCV x 100). Serum biochemical indices such as total protein were determined using the biuret method while the glucose oxidase method by Christensen [18] was used to analyze the glucose content. The serum enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using the method of Reitman and Frankel [19].

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 influence of the diets on the lipid of the fresh and stored eggs. The lipid profile of the fresh eggs 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 not differ from the T_1 (control) and T_4. The total cholesterol (TC), triglyceride and LDL (low density lipoprotein) were found similar between the groups.

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 total 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.

Table 1. Influence of treatment on the lipid of the fresh and stored eggs

Parameter (mmol/L)	T_1 (0%)	T_2 (0.5%)	T_3 (1.0%)	T_4 (1.5%)	SEM
Fresh eggs					
Total Cholesterol	25.00	24.33	24.33	23.66	0.22
Triglyceride	6.20	6.20	6.16	6.20	0.02
HDL	9.30^{a}	6.53^{ab}	5.83^{ab}	3.46^{b}	**0.77**
LDL	0.16	0.10	0.13	0.13	0.10
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, SEM= standard error of mean

Table 2 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 Banerjee [20] 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.

From the results of the serum biochemistry of layers fed the varying percentages of the leaf meal in Table 3, 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, total cholesterol, triglyceride and low-density lipoprotein were significantly ($P < 0.05$) different. The total protein, total 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

Table 2. 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
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

Table 3. 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
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

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.

4. DISCUSSION

The reduction in HDL as the percentage of the leaf in the diet increased and the absence of differences in total cholesterol, triglyceride and LDL of the eggs tallied with the report of El-Sheikh et al. [21] 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 Lala et al. [22] who reported an elevation in the HDL, and reduction in LDL and total cholesterol in the eggs of layers fed with moringa leaf meal. According to Sembuligam and Sembuligam [23] HDL helps to transport cholesterol to the liver for its excretion. It was established by Boden [24] and Rahilly-Tierney et al. [25] 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 total cholesterol, triglyceride and LDL of the fresh eggs indicated that the varying percentages of moringa leaf meal did not affect the parameters when the egg was still fresh.

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 7^{th} 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 [24,25]. This result tallied with the report o El-Sheikh et al. [21] who found significant increase in high density lipoprotein (HDL) in serum from all treated groups and in the yolk when moringa leaf powder (MOLP) was included at levels of 1.5 and 2 g/kg diet. However, the lower levels of the HDL in T_2 – T_4 at the end of the 14^{th} day (second week) of storage with the corresponding higher level of LDL in T_2 will not be good health. The absence of variations in the HDL and LDL coupled with the decreasing trend of the total cholesterol and increasing trend of the triglycerides after the third week of storage could be as a result of the effect of the treatments. This tend to agree with Fahey [26] who stated that moringa is a vital source of phytochemicals (natural plant chemicals) such as glucosinolates, alkaloids and isothiocyanates that provide cholesterol lowering activities. It supported the finding of Ghasi [27] that moringa leaf has hypocholesterolemic properties.

The reducing levels of the Hb, PCV, MCH and MCV as the percentage of moringa meal in the feed increased supported the finding of Aderinola et al. [28] who 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 Kuttappan [29] when 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 when the animal has not shown any signs of illness [30]. 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 results obtained for AST were not different from the report by Makanjuola et al. [31] while the ALT tallied with those of Hassan et al. [32]. The variation observed in the total protein level tallied with the report of Hassan et al. [32] who reported difference in total protein of the blood in broilers that were fed the same leaf meal. According to Gregg and Lightfoot [33] 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, total 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, total 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 Teteh et al. [34] 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 Aderimola et al. [28] who studied the effect of moringa in broiler birds. This finding was in line with Zanu et al. [35] 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.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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