



Laying Performance, Yolk Cholesterol, Serum Lipid Profile and Haematological Response of Layer Hens Fed Diet Containing Moringa Leaf Meal and Garlic Powder as Feed Additive

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ABSTRACT

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The aim of the research was to determine the laying performance, yolk cholesterol, serum lipid profile and haematological response of hens fed a diet containing moringa leaf meal and garlic powder as feed additives. A total of 220 (18 weeks) Isa brown hens weighing 1.91-1.94 kg at the point of first lay were allotted to 4 treatment groups in a completely randomized design (CRD) with 5 replicates of 11 birds each. Treatments were as follows: T1: 0 g moringa leaf meal + 0 g garlic powder kg⁻¹, T2: 0 g garlic powder +50 g moringa leaf meal kg⁻¹, T3: 50 g garlic powder+ 0 g moringa leaf meal kg⁻¹, T4: 50 g moringa leaf meal +50 g garlic powder kg⁻¹. From the results, hen day egg production improved significantly higher in treatment 3 compared to other treatment groups. Treatment 4 had the highest ($p<0.05$) value for egg weight among the treatments. Yolk cholesterol, serum cholesterol, serum triglyceride and low-density lipoprotein values were higher ($p<0.05$) in T1 compared to treatment groups. High density lipoprotein values of T2 and T4 were the same ($p>0.05$), but higher ($p<0.05$) than the values recorded in T1 and T3. Haematological values such as RBC and WBC improved significantly in the treatment groups compared to control group (T1). This showed that the immunity of the birds was not compromised. In conclusion, since, hen day egg production reduced significantly with the combination of moringa and garlic, it was recommended that T3 can be included in diet of hens for improved laying performance.

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INTRODUCTION

Despite the contribution of poultry to human protein need in form of eggs and meat, poultry is being faced with a number of issues, such as high rate of mortality and decrease in egg production. Farmers who used to depend on the use of antibiotics in the time past for disease control and prevention are now facing challenges in disease control and prevention during production. This is a result of the prohibition placed on the utilization of antibiotics by the European Union in 2006 (Choi, 2018). Nevertheless, antibiotics are utilized during farm animal production for the prevention of pathogenic invasion and control of diseases and thereby increasing the production of meat, eggs, and feed efficiently (Hassan et al., 2018). European Union decided to place ban on the use of antibiotics as a result of the amplified issues that are connected with their resistance in farm animals and residues in meat (Carvalho and Santos, 2016). Common issues brought on by the use of dietary antibiotics include the emergence of resistant bacteria and an imbalance in the natural microbiota (Hrnčár et al., 2017). Foods have been found to be affected negatively by the usage of antibiotic growth promoters such penicillin, tetracycline, and amphenicol (Diarra and Malouin, 2014). This is also true of beta-agonists like clenbuterol, which can result in tachycardia, palpitations, muscle tremors, and food poisoning (Hoffman et al., 2001).

However, animal producers are now looking for natural additives that will serve as alternatives to antibiotics because of the ban on the use antibiotics by the European Union (Abd El-Hack et al., 2022). In farm animal feeding, several substitutes to antibiotics growth promoting agents have been proposed. These include: organic acids (Capcarova et al. 2014), prebiotics, probiotics and phytogetic additives (Wang et al., 2015; Ri et al., 2017; Ahsan et al., 2016; Mashayekhi et al., 2018; Abouelezz et al., 2019), sodium butyrate and rosemary powder (Alagawany et al., 2019), bee products (Haščík et al., 2020), enzymes (Al-Harathi, 2006) and other natural products (Hrnčár et al., 2018).

Some plant-based materials have been added to chicken diets (Sugiharto, 2016) to serve as natural antibiotics, immunomodulatory agents, and growth promoters for birds. *Moringa oleifera* leaf powder is one of them. According to research findings *moringa oleifera* plant has long been recognized for its high nutritional content and useful qualities, such as antimicrobial activity (Mune et al., 2016). Numerous initiatives have been launched to thicken eggshells and lower cholesterol levels in eggs because excessive cholesterol levels in egg yolks can discourage consumers from buying and eating eggs. Egg yolk cholesterol reduction will improve both public health and poultry sector (Mahmoud et al., 2010; Meliandasari et al., 2015). According to nutritional assessments of *moringa* by González-Burgos et al. (2021), *moringa* is composed of 1.108 mg β -carotene, 25.02% proteins, 10.42% fat, 15.2 mg/100 g vitamin C, 11.83% dietary fiber, 326.4 μ g/100 g vitamin B1 and 28.50% carbohydrates.

Another phyto-genic additive or plant herbs that have been used to replace antibiotics in poultry nutrition is garlic powder. Garlic plays a very crucial role in enhancing efficiency of nutrient digestibility, immunity and health of birds (Kumar et al., 2010). Garlic has antimicrobial antioxidant properties and can reduce mortality in birds (Sivam 2001; Stanačev et al., 2010). According to literature, garlic has been observed as a performance and egg quality boosting agent in laying hens when included in the diet (Lim et al., 2006; Yalcin et al., 2006). Because of the urgent need to discover a safe alternative material that will replace antibiotics in poultry feed, this present study was thus, designed to determine the laying performance, yolk cholesterol, serum lipid profile and haematological response of layer hens fed diet containing moringa leaf and garlic powder as feed additive.

MATERIALS AND METHODS

Ethical Consideration

The research was done in line with the ethical provisions of the Committee (No: OSE389SORRY02.17.11.2022) on the use of farm animals for research at the University of Nigeria, Nsukka.

Study Site

The study took place at the Teaching and Experimental Farm, Avian Section of the Department of Animal Science of the University of Nigeria, Nsukka. The study area lies within longitude 6° 45'E and 7° E and latitude 7° 12.5 'N and at an altitude of 447m above sea level. The annual rainfall according to the Metrological Center, Crop Science Department, University of Nigeria, ranges from 1567.05mm-1846.98mm. The study lasted for 12 weeks.

Experimental Birds and Management

A total of 220 (18 weeks) Isa brown hens weighing 1.91-1.94 kg at the point of first lay were allotted to 4 treatment groups in a completely randomized design (CRD) with 5 replicates of 11 birds each. Treatments were as follows: T1: 0 g moringa leaf meal + 0 g garlic powder kg⁻¹, T2: 0 g garlic powder +50 g moringa leaf meal kg⁻¹, T3: 50 g garlic powder+ 0 g moringa leaf meal kg⁻¹, T4: 50 g moringa leaf meal +50 g garlic powder kg⁻¹.The hens were housed in a deep litter system that was demarcated with good net system. Feed and clean water were made available continuously throughout the feeding trial using good feeders and plastic watering cans placed in strategic places in the experimental house. Thermometer was used to monitor the temperature in the trial room. Nest boxes were constructed using clean and quality wooding planks. Birds were checked twice daily for mortality. Proper vaccination programs for laying flocks were followed. Eggs were picked two times daily form each replicate.

Experimental Diet

The trial diet and its chemical constituents are exhibited in Table 1. According to NRC (1994) nutrient requirements for laying birds, the diet used was formulated. Using AOAC (2012) chemical compositions of the diet without inclusion of additives (moringa and garlic) used was evaluated. The additives used in the current study were not part of diet formulation.

Table 1. Percentage composition of the experimental diet diets

Ingredients (%)	Quantity
Maize	40.00
Wheat offal	22.00
Palm kernel cake	12.00
Soyabean meal	14.00
Oyster shell	6.00
Pail oil	2.00
Bone meal	3.00
Lysine	0.25
Methionine	0.25
Salt	0.25
Vitamin premix	0.25
Total	100
Calculated composition (%)	
Crude protein	16.20
Metabolizable energy (Mcal/kgME)	2560.00
Chemical compositions (%)	
Crude fiber	6.10
Ether extract	3.80
Dry matter	88.00
Ash	9.31
Moisture	12.00
Crude protein	16.19
Nitrogen free Extract	52.70

Each 2 kg of vitamin premix contains: vitamin A: 1000000 IU; vitamin D3: 2200.000 mg; vitamin B1: 1500 mg; vitamin B2: 5000 mg; vitamin K3: 2000 mg; vitamin B12: 10 000mg; vitamin B6: 1500 mg; vitamin E: 10000 mg; Biotin: 20 mg; Niacin: 15000 mg; Folic acid: 5000 mg and Calpan: 5000 mg.

T1: 0 g garlic +0 g moringa leaf meal/kg, T2: 0 g garlic powder+50 g moringa/kg, T3: 50 g garlic powder+0 g moringa leaf meal/kg and T4: 50 g garlic powder +50g moringa leaf meal/kg.

Data Collection

Performance Indices

An electronic weighing scale of 6 kg capacity was used to take the initial body weights of the birds before the study started. Each day throughout the study, hen-day egg production was computed as follows: total eggs collected / total live hens in each replicate. The average weight of eggs laid was calculated as follows: Total weight of eggs / number of eggs. The daily feed intake was calculated by dividing the difference between the feed delivered the day before and the leftover feed in the following morning. The average daily feed intake was calculated by dividing the total kg of feed consumed by the birds by the number of days the feeding trial lasted.

Determination of Serum Lipid Profile and Egg Cholesterol

For the determination of egg yolk cholesterol, 5 eggs were randomly selected on weekly basis from each replicate. They were gently cracked and mixed together. Then, 5 ml of the mixed egg were used for the analysis of yolk cholesterol. At 1800r/m, the mixed egg samples were centrifuged and yolk cholesterol was analyzed using Hitachi 902, Auto lipid Analyzer. Furthermore, at the end of the feeding trial, blood samples were collected from 3 randomly selected hens from each replicate for serum cholesterol, high density lipoprotein, low density lipoprotein and triglyceride determination employing chemistry auto analyzing kit, using appropriate enzymatic methods.

Hematological Indices

At the end of the study, one hens of comparable weights were selected randomly from each replicate after which 3mls of blood samples were collected from them using sterile needles. The blood samples were emptied into a well labeled sterilized bottle containing EDTA for haematological examination. Packed cell volume and hemoglobin concentration were assessed using protocols of Mitruka and Rawnsley (1977). Red blood cell and total white blood cell counts were examined with the help of automated Idexx Vet Test Chemistry Analyzer (IDEXX Laboratories, Inc).

Statistical Design and Analysis

By application of Analysis of variance (ANOVA) contained in the statistical package (SPSS, 2014), data generated were analyzed. Mean differences were separated by using Duncan's New Multiple Range Test (Duncan, 1955) as delineated by Obi (2002). The statistical model applied was stated below:

$$X_{ij} = \mu + T_i + E_{ij}$$

X_{ij} = individual observation

μ = population mean

T_i = treatment effect

E_{ij} = experimental error

RESULTS

Laying Performance

Table 2 and Figure 1-2 show the results of the effect of moringa and garlic powder on layer hen performance. From the results, the values for initial body weight and average daily feed intake were not among were not significant ($p>0.05$), while the values for hen day egg production and egg weights were significant ($p<0.05$). Hen day egg value of T2 and T4 were the same ($p>0.05$), but lower than the values of 78.75 and 83.30 recorded in T1 and T3 respectively. Egg weight values of T4 was the highest among the treatments.

Table 2. Effect of moringa leaf meal and garlic powder of layer performance

Treatments	IBW (kg/bird)	ADFI (g/bird)	HDEP (%)	Egg weight (g)
T1	1.92	1210.13	78.75b	60.99b
T2	1.93	1190.72	65.87c	59.53b
T3	1.94	1184.20	83.30a	56.39c
T4	1.91	1168.70	66.69c	62.61a
SEM	0.37	11.41	2.10	0.39
P-values	0.25	0.76	0.04	0.03

a-cMeans on the same row with different superscript are significantly different ($p<0.05$), SEM=Standard error of the mean, IBW= Initial body weight, ADFI= Average daily feed intake, HDEP= Hen Day egg production, T1:0g garlic +0 g moringa leaf meal/kg, T2: 0 g garlic powder+50 g moringa/kg, T3: 50 g garlic powder+0 g moringa leaf meal/kg and T4: 50 g garlic powder +50g moringa leaf meal/kg.

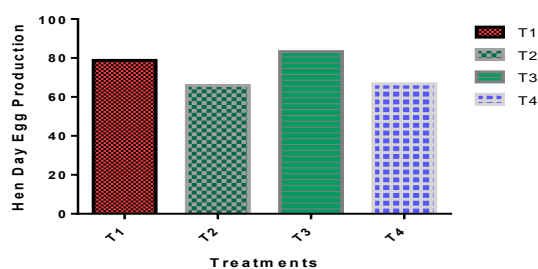


Figure 1. Effect of moringa leaf powder and garlic powder on hen day egg production

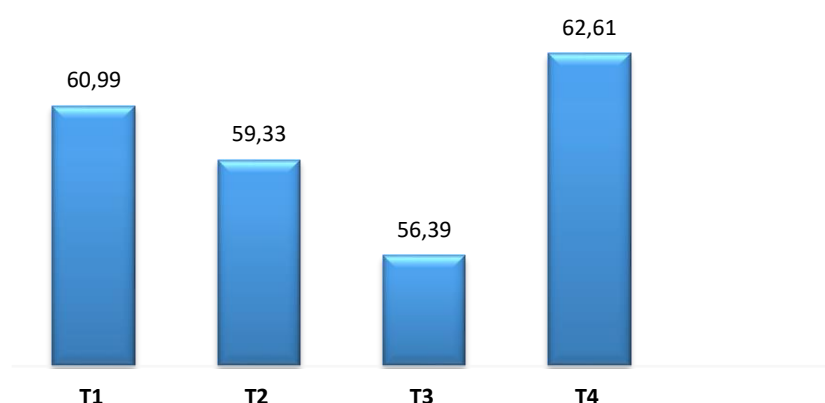


Figure 2. Effect of moringa leaf meal and garlic powder on egg weights

Serum Lipids and Yolk Cholesterol

Results of the effect of moringa and garlic powder on serum and egg lipid profile of layer hen are shown in Table 3 and Figure 3. Values of serum cholesterol values of T3 and T4 are the same ($p>0.05$), but significantly lower than the value recorded in T1 birds and higher than the value recorded in T3. Serum cholesterol and triglyceride values of T2, T3 and T4 were the same ($p>0.05$), but lower than the values of 141.13 and 113.13 observed for T1 birds for serum cholesterol and triglyceride. Values of low-density lipoprotein value T2 and T3 were the same ($p>0.05$), but lower than values of 106.49 and 80.43 recorded in T1 and T4 respectively. High density lipoprotein values of T1 and T3 were the same ($p>0.05$), but lower than values of 83.75 and 78.88 observed for birds on T2 and T4 that were also the same ($p>0.05$).

Table 3. Effect of moringa leaf and garlic powder on serum and egg yolk cholesterol

Treatments	Yolk cholesterol (mg/dl)	Serum cholesterol (mg/dl)	Serum triglyceride (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
T1	145.25a	141.13a	113.00a	106.49a	66.83b
T2	124.63b	100.38b	87.75b	55.21c	83.75a
T3	85.38c	98.37b	88.75b	65.85c	68.61b
T4	129.63b	107.63b	95.87b	80.43b	78.88a
SEM	19.23	13.76	5.99	5.5	5.7
P-values	0.02	0.05	0.03	0.05	0.04

a-c Means on the same row with different superscript are significantly different ($p<0.05$), SEM=Standard error of the mean, HDL= High density lipoprotein, LDL=Low density lipoprotein, T1:0g Garlic +0 g moringa leaf meal/kg, T2: 0g garlic powder+50 g moringa/kg, T3: 50 g garlic powder+0 g moringa leaf meal and T4: 50 g garlic powder +50g moringa leaf meal/kg.

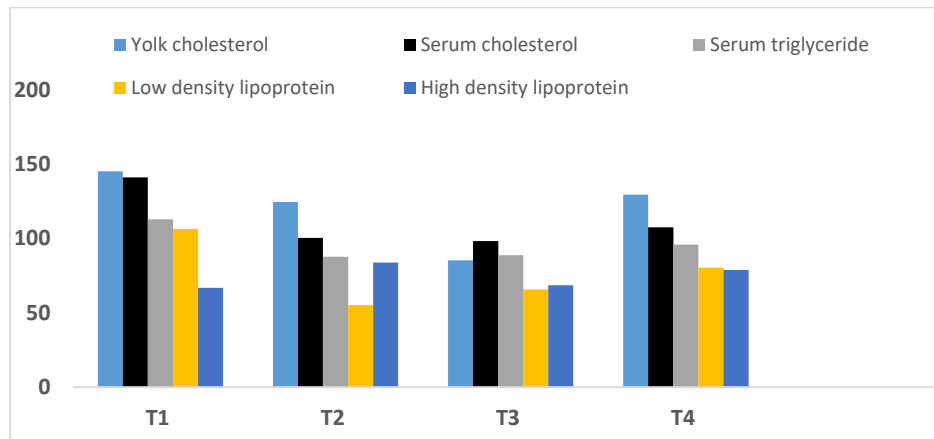


Figure 3. Effect of moringa leaf meal and garlic powder on serum lipids and of yolk cholesterol

Results of the effect of moringa and garlic powder on haematological characteristics of layer hen are presented in Table. Values of haemoglobin, lymphocytes and monocytes were not significant ($p>0.05$), while values of packed cell values, red blood cell counts, white blood cell counts, neutrophils and basophils were significant ($p<0.05$). Packed cell volumes of T1, T2 and T3 were the same ($p>0.05$), but lower than the value of 34.75 observed for birds on T4. Red blood cell counts values of T3 and T4 were the same ($p>0.05$) and the highest among the treatments followed by values in T2 and T1. White blood cell counts of T1 and T3 were the same, but lower than the values recorded in T2 and T4. Neutrophil values were not significant ($p>0.05$) among the treatments. Basophil values of T1 and T4 were the same ($p>0.05$), but lower than values of 0.25 and 0.24 recorded in T2 and T3.

Table 4. Effect of moringa leaf meal and garlic powder on haematological characteristics of layer hen

Parameters	T1	T2	T3	T4	SEM	P-values
PCV (%)	29.00b	27.25b	30.88ab	34.75a	0.90	0.05
Hb (g/dl)	8.78	9.63	9.08	9.03	0.25	0.21
RBC($10^6/\mu\text{l}$)	7.35d	8.45c	10.45a	9.51a	0.48	0.04
WBC($10^6/\mu\text{l}$)	9912.50b	11193.75a	10675.00b	11281.25a	411.55	0.02
Neutrophils(%)	27.87	31.38	29.37	28.87	1.43	0.18
Lymphocytes(%)	67.13	68.88	70.13	69.88	11.54	0.43
Monocytes(%)	13881	0.75	0.75	1.00	0.29	0.12
Basophils(%)	0.88a	0.25b	0.24b	1.38a	0.25	0.01

a-c Means on the same row with different superscript are significantly different ($p<0.05$). SEM=Standard error of the mean, PCV= Packed cell volume, Hb= Haemoglobin, RBC= Red blood cell, WBC= White blood cell, T1:0 g garlic +0 g moringa leaf meal/kg, T2: 0g garlic powder+50 g moringa leaf meal/kg, T3: 50 g garlic powder+0 g moringa leaf meal/kg and T4: 50 g garlic powder +50g moringa leaf meal/kg.

DISCUSSION

Results of the effect of moringa and garlic powder on performance of laying hens are shown in Table 2 and Figure 1-2. There were no significant differences ($p > 0.05$) in feed intake values among the treatments. This shows that *Moringa oleifera* does not have a toxic effect or contains factors that limit intake in laying hens as reported by Ekayuni et al. (2017) and Saini et al. (2014). Even though values of feed intake among the treatments were non-significant, but numerically, the control had the highest value for feed intake. This demonstrates that the strong odor of garlic may have acted as a deterrent to feed intake in the treatment groups. Qureshi et al. (1983) discovered that the distinct odor of garlic caused birds' feed consumption to drop when garlic was added to their diets of birds. The results also corroborated by Ayssiwede et al. (2011) who found that hens feed intake decreased when 24% of moringa leaf meal were added to the diet of birds. However, the combination of garlic powder at 50g/kg and moringa at 50 g/kg produced a decrease ($p < 0.05$) in egg production. When compared to T1 (control) and other treatment group, the inclusion of garlic powder (50g /kg) as a single feed additive in T3 increased egg production (83.30). This concurs with the findings of Canogullari et al. (2009) who showed that egg production increased in the treatment groups supplemented with 0.5% and 1.0% garlic powder in comparison to the control group. However, as reported by (Prasad and Ganguly 2012), the presence of phytochemical compounds contained in garlic, may be the cause of the increased hen day egg production recorded in T3. Bidura et al. (2017) noted that laying hens' egg production increased after receiving herbal extracts (*Sauropus* and garlic leaves) in drinking water. Hen day egg production values decreased ($p < 0.05$) in moringa supplemented group (T2 and T4) when compared with control and T3. The results agree with Olugbemi et al. (2010) and Abdelnour et al. (2018) who found that using moringa leaf meal at a rate greater than 10% resulted in negative effects, possibly as a result of an increase in the anti-nutritional factors and dustiness of moringa as well as low energy and protein digestibility. The current result contradicts the findings of Siti et al. (2017), Suarjana et al. (2018), and Mohammed et al. (2012) who showed that moringa leaves increased egg production in laying hens. Based on the results of the current study, it might be scientific to conclude that the combination of garlic powder and moringa leaf meal in T4 may have been responsible for the reduced egg production when compared to other treatment as a result the increased antinutritional factors in moringa leaves. Value for egg weight, which was highest in the same T4 that got combination of garlic powder and moringa, reduced considerably in T3 recipients of just garlic powder at 50 g/kg. According to Kwariet et al. (2011), garlic powder had no appreciable effects on the egg weight of Vanaraja laying hens. Compared to other treatments, egg weight increased when moringa and garlic were combined in T4 could be related to the synergistic effect of phytochemicals found in both garlic and moringa.

As stated by Prasad and Ganguly (2012) phytochemical substances found in garlic, may be related to the increase in egg weight in combination with moringa in T4. This could be also as a result of various compounds contained in moringa leaves such as vitamin A, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, ascorbic β -carotene, calcium, iron, and α -tocopherol. According to Anwar et al. (2005) the leaves of *Moringa* plant are high in carotenoids, flavonoids, and vitamin C. Additionally, increased egg production and weights in favor of the treatment groups may also be attributable to the digestive benefits of moringa in birds. Supplements containing plant extracts have been shown to improve the digestibility of nutrients in the digestive system of birds (Hernandez et al. 2004; Nkukwana et al. (2014). So better egg production and weight in favor of the treatment groups could be as the result of increased feed digestion and nutrient absorption caused by the moringa and garlic supplementation. According to Ramakrishna et al. (2003), herbal extracts such as garlic can increase the activity of pancreatic enzymes and create favorable micro environmental conditions for better nutrient utilization in mice. Final body weight across the treatments were not significant ($p>0.05$). According to Toghyani et al. (2011), broilers' ultimate body weights and feed consumption were not affected by the inclusion of garlic powder at levels of 2 and 4% in their diets. According to Milošević et al. (2013) feeding broiler chickens 1.5% and 3% garlic powder had no impact on their final live weight or feed consumption (Abbas 2013).

In Table 3 and Figure 3, the effects of moringa and garlic powder on serum lipid profiles and egg yolk cholesterol are shown. According to the findings, egg yolk cholesterol levels were lowered by the combination of moringa leaf and garlic powder in feed as well as when used separately. Similar findings were reported by Sharma et al. (1979), who found that feeding garlic powder to laying hens for three weeks lowered the cholesterol in their egg yolks by 4.1 or 5.5%. The findings of the current investigation unequivocally demonstrate that garlic powder has a lowering effect on the cholesterol levels in egg yolks. According to Wibawa et al. (2016) using herbal ingredients such as garlic can lower yolk cholesterol. However, the decreased serum and yolk cholesterol observed in favor of the treatment groups may in part be attributable to a decline in the activity of the lipogenic enzyme and an increase in the excretion of bile acids through the feces as a result of the carotene concentration in moringa leaves. The hydroxymethyl glutaraldehyde-CoA enzyme is involved in the formation of cholesterol, hence the potential of β -carotene to lower cholesterol is correlated with the lowering of this enzyme. Additionally, the fiber in *M. oleifera* plays a crucial function in binding and elimination of cholesterol, which might be result in a drop in egg cholesterol levels (Oladunjoye et al., 2010; Ghasi et al., 2000; Olugbemi et al. 2010a). Furthermore, treatment groups had considerably decreased serum levels of triglycerides, cholesterol, and low-density lipoprotein (T1-4). Similar to the results was Azeke and Ekpo (2008) who reported that diet supplementing with garlic powder at levels of 1% and 2% in birds decreased LDL. In contrast, Lim et al. (2006) observed that HDL-cholesterol was not affected when the layers were fed 0%, 1%, 3%, and 5% garlic

powder for 5 weeks and increased high density lipoprotein levels were observed in the treatment groups. The presence of alkaloids in moringa leaf meal may be the cause of the decrease in serum triglyceride, cholesterol, and low-density lipoprotein levels (Chen et al., 2003). The current study's findings are consistent with those of Aghazadeh et al. (2011) and Rafiee et al. (2013), who found a decrease in triglyceride levels in broiler hens given thyme mint and ginger+thyme mixes respectively. Abdul et al. (2012) and Awodola-Peters and Yahaya (2017) who observed a significant increase in the high-density lipoprotein level of broiler chickens given various herbal infusions and graded levels of roselle calyx was comparable with the results of the current study. The findings of Nihad et al. (2017) who observed that supplementing broiler chicken's diets with moringa oleifera leaf powder significantly increased their serum high-density lipoprotein levels are also in consistent with the findings of the current study. Table 4 and Figure 4-5 display the findings of the effect of moringa and garlic powder on the haematological response of hens fed diet containing garlic powder and moringa leaf meal. The haematological values in all the treatment groups are within the range typical for avian species. This was an indicating that the birds were in good health. According to research, moringa and garlic powder, particularly moringa, provide enough amounts of high-quality dietary iron and protein, which can lead to ideal concentrations of blood components. Iron is present in moringa oleifera leaf at (250, 5 dpm, 490 mg/kg) iron. Even though haemoglobin values were not significantly different across the treatments, treatment groups having numerically higher haemaglobin values may have caused by higher iron consumption contained in moringa which caused an improvement in haemoglobin synthesis. Red blood cell values among the treatments were within acceptable limits (McDowell, 2003). White blood cell counts considerably increased in treatment groups compared to control groups. This could as a result of the garlic and moringa inclusion in the diet of the hens. Garlic and moringa contains various phytochemicals used for building strong immunity and disease fighting abilities in farm animals. Similar to Gupta et al. (2006) mice given moringa oleifera leaves extract showed a substantial rise in white blood cell count.

CONCLUSION

From the results of this study, the utilization of garlic and moringa leaf powder had a positive effect on the bird's egg quality characteristics, haematological and lipid profile traits. Treatment groups had the highest improvement in the egg quality, haematological and lipid profile traits determined. Since desire of most farmers to have more eggs for sale, treatment 3 (50 g garlic/kg) was recommended for use by poultry farmers for improving hen day egg production in laying birds.

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Conflict of interest

The authors declare no competing interest.

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Authors Contribution

The authors contributed equally to the article.

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