Haematology, Serum Biochemistry and Egg Lipid Profile of Laying Hens Fed Diets Supplemented With Moringa Oleifera and Senna Occidentalis Leaf Meal Composite Mixture.

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Abstract **:A feeding trial was carried out to assess the effect of Moringaoleifera leaf meal (MOLM) and Senna occidentalis leaf meal (SOLM) composite mixture on their haematology, serum chemistry and egg lipid profile of total of 150 Isa brown layers of sixteen weeks of age. Thirty (30) chicks were randomly selected and allocated to each of the five (5) treatment groups (T_1, T_2, T_3, T_4 and T_5) in a completely randomized design (CRD) in a battery cage system. Each treatment group contained three (3) replicates with ten (10) birds per replicate and the feeding trial lasted for ten (10) weeks. The experimental treatment included the control (maize – soya bean meal basal diet with oxytetracyline administered to the birds through drinking water) as T_1, T_2 (same basal diet was mixed with 0.5% MOLM + 2.0% SOLM), T_3 (same basal diet was added with 1.0% MOLM + 1.5% SOLM), T_4 (same basal diet was added with 1.5% MOLM + 1.0% SOLM) and T_5 (same basal diet was mixed with 2.0% MOLM + 0.5% SOLM) and T_2 to T_5were without antibiotic in the water. MOLM and SOLM blend significantly ($P<0.05$) affected all the haematological and serum biochemical indices measured except for creatinine values that were not affected. Total cholesterol, triglyeride, low density lipoprotein (LDL) and very density lipoprotein (vLDL) values of eggs laid by the hens were reduced significantly ($P<0.05$) but high density lipoprotein (HDL) values were increased significantly ($P<0.05$) by the supplementation of MOLM and SOLM mixture compared to the control group. Therefore, a mixture of 2.0%MOLM and 0.5%SOLM could be included in the diets laying hens without any adverse effect on the blood profileand to improve the lipid profile of eggs as well as an alternative to antibiotics in laying hens.**

Key words: **Antibiotics, haematology, layers, lipid profile, serum chemistry**

I. INTRODUCTION

Poultry production is one of the most popular livestock enterprise adopted by small and medium scale farmers in both rural and urban areas as it offers the highest turnover rate and quick return on investment amongst the livestock farming options (Afolayan et al., 2014). Even though the benefit of poultry production is being eroded by high cost of feed and diseases, there had been an exponential increase in the productivity of modern poultry stocks of both meat and egg types of the global poultry industry in the last few decades (Omoikhoje et al., 2018b). This improvement in poultry production can be ascribed to synergies between the advances made in major activities of poultry management, housing, nutrition and ration formulation, breeding and genetics as well as better diagnosis and control of avian diseases. Amongst all these core elements, poultry health and diseases are the least predictable. In layers production for instance, good health accompanied with fast growth is necessary if maximum productivity is to be achieved. Although high quality and adequate quantity of feed may be provided, the amount of feed digested, nutrients absorbed and utilized are very important. Generally, digestion in poultry may among other factors depend on the micro-organisms that naturally inhibit and colonize the digestive tract (Ndelekwute, 2011). Thus, the need to formulate diets that enhance gut health and function has become imperative. Consequently, feed additives such as antibiotics have been used for these purposes at sub-therapeutic doses in poultry diets. They act directly against pathogens in the gut creating a favourable environment for protein and energy digestion, absorption and metabolism (Puyalto and Mesia, 2002). However, birds raised with these feed additives achieve good performance but the potential side effects such as host and cross drug resistance had been a public health concern globally (Al-Harthi, 2006) and this has led to the ban of these products by many countries of the world (Kehinde et al., 2011). Against this background, research efforts by Nutritionists have been geared towards the search for plant materials and their derivatives as natural antibiotics and probiotics in animal feeds (Cogliani et al., 2011). However, a wide range of drugs have been currently employed in the management of hepatic disorders and other diseases in livestock, but alternative approaches from the traditional medical systems have been used (Maeda-Machangu et al., 1997). Up to one-third of all commercial swine and chicken rations in Europe now use mixtures of herbs and spices to accelerate growth and maintain health (Odoemelan et al., 2013). Some of these herbs and spices indigenous to Africa enhance nutrient utilization and performance of broiler chickens (Omoikhoje et al.,2018b).

Bioactive secondary metabolites of plants, such as carotenoids, phenolic compounds, polyphenols, flavones, flavonoids, alkaloids, polypeptides and essential oils have been shown to have anti-bacterial, anti-fungal, anti-aging, anti-oxidant and functional properties (Cowan, 1999). In particular, it had been demonstrated that essential oils such as cinnamaldehyde, eugenol, thymol, and carvacrol have anti-bacterial action against multiple pathogenic bacteria (Hernandez et al., 2004). Sharma et al. (2016) opined that the antibacterial properties and immunomodulatory effects of the bioactive compounds in green leaves may be ascribed to the therapeutic effects of the leaves. In the light of this, Prakoso et al. (2018) asserted that the use 5% of *Sauropus androgynus* leaf meal improved the final live weight of broiler chickens contaminated with aflatoxin. The utilization of 1% *Eucalyptus camaldulensis* leaf meal to improve the sensory evaluation such as flavour and juiciness of thigh and breast meats of broilers as well as the reduction of hardness and thiobarbituric acid reactive substances in the thigh and breast meats of the birds compared to control have been reported (Mustafa, 2019).

Moringa oleifera is rich in bioactive compounds and may be a potential candidate as phytogenic feed additive (Melesse et al., 2011). Moringa leaves contain vitamins, flavonoids and carotenoids, which not only serve as essential nutrients, but also enrich poultry meat and eggs, and intensify the pigmentation of the shanks and egg yolk (Fasuyiet al., 2005). These enriched eggs can be marketed as designer eggs or functional foods. The leaves are good sources of digestible protein, β-carotene, vitamin C, calcium, iron and potassium (Okiki et al, 2015). Moreover, toxic heavy metals such as mercury, arsenic and cadmium are absent from the leaves of *Moringa oleifera* making it safe as leaf meal that can be added into feed for poultry (Donkor et al., 2013). *Moringa oleifera* leaves had been reported (Okiki et al.,2015) to contain 7.88, 28.00, 3.88, 9.82, 12.57 and 37.87% of moisture, crude protein, ether extract (fat), crude ash and carbohydrate respectively.*Senna occidentalis* on the other hand alsoknown as coffee weed had been found to possess significant anti-bacterial, anti-fungal, anti-diuretic, anti-allergic and anti-inflammatory properties. The seed is a febrifuge and sedative, infusion is taken to calm one's nerves and as treatment for kidney problems, haemorrhage, worms and cleaning of womb and tube(Sambasivam et al., 2016). Omoikhoje et al. (2018a) reported 9.35, 21.88, 19.72, 16.88, 9.70 and 22.47% of moisture content, crude protein, crude fibre, crude fat, ash and nitrogen-free extract (NFE) respectively in *Senna occidentalis* leaves. The authors also reported the presence of cardiac glycosides, saponins, phenols, flavones, flavonols and alkaloids in dried *Senna occidentalis* leaf meal. In poultry, *Senna occidentalis* aqueous leaf extract have been used as probiotic additive to improve performance, carcass traits and better cost and returns in broiler chickens (Omoikhoje et al., 2018b). The thrust of this study therefore is to assess the effect of *Moringa oleifera* and *Senna occidentals* leaf meal composite mixture on the haematology, serum chemistry and egg lipid profile of laying hens.

II. MATERIALS AND METHODS

Experimental location and climate

This experiment was conducted at the Poultry Unit of the Livestock Teaching and Research Farm, Ambrose Alli University, Ekpoma, for a period of eight (8) weeks (between the months of June and August, 2019). The farm is located in the tropical savannah rain forest vegetation belt in Nigeria with longitude 6.44^{o}N and 6.08^{o}E, with a mean ambient temperature of about 26^{o}C in December to 34^{o}C in February with an average relative humidity of 61% in January and 92% in August with a yearly average of about 82%. The vegetation in this area represents an interface between the tropical rain forest and the derived savanna.

Sources of ingredients, fresh Moringa oleifera and Senna occidentalis leaves

The ingredients for the layer diets were purchased from a reputable feed dealer in Benin City, Edo State, Nigeria. Fresh *Moringa oleifera* and *Senna occidentalis* leaves were harvested within the University community.

Processing of Moringa oleifera and Senna occidentalis leaves

The leaves of *Moringa oleifera* and *Senna occidentalis* were cleaned of dirt and sparsely spread on jute mat at room temperature for 6-7 days until they became crispy. The leaves were turned regularly to avoid uneven drying and decay and also to maintain the greenish colour of the leaves. The dry crispy leaves were hammer milled and passed through a 2mm sieve and then stored in airtight plastic containers to avoid absorption of moisture till it was used for laboratory analyses and preparation of the experimental treatments as supplement to the basal diet. The *Moringa oleifera* leaf meal and *Senna occidentalis* leaf meal were designated as MOLM and SOLM respectively.

Feeding and experimental treatments

The birds were fed commercial diets for two weeks acclimatization period. Thereafter, they were fed with formulated maize soyabean basal layer diet (Table 1). The treatment groups included: the control (maize-soya bean meal basal diet but the birds took the antibiotic (Oxytetracycline) in drinking water) as T_1, T_2 (same basal diet was mixed with 0.5% MOLM + 2.0% SOLM), T_3 (basal diet was added with 1.0% MOLM + 1.5% SOLM), T4 (basal diet was added with 1.5% MOLM + 1.0% SOLM) and T_5 (basal diet was mixed with 2.0% MOLM + 0.5% SOLM). The antibiotic (oxytetracycline) was administered to the birds in the control group at weekly intervals following the manufacturer's prescription as a positive control (T_1). The MOLM and SOLM blend were used as additive to the basal diet and were without antibiotic in the drinking water. The basal diet was prepared and formulated to meet the nutrient requirements of broiler chickens (NRC 1994).

Experimental birds, housing design and management

A total of 150 Isa brown layers of sixteen weeks of age were used for the experiment. On arrival, thirty (30) chicks were randomly selected and allocated to each of the five (5) treatment groups (T_1, T_2, T_3, T_4 and T_5) in a completely randomized design (CRD) in a battery cage system. Prior to this, the birds were given vitamins and ant-stress to ease the stress due to the transportation. Each treatment group contained three (3) replicates with ten (10) birds per replicate. Thereafter, the birds were allowed free access to the dietary treatments throughout the duration of the study. All routine management practices were carried out including medication, vaccination against gumboro and Newcastle diseases, cleaning of feeders and drinkers as well as regular change of litter materials.

Blood Sample Collection and analyses

At the end of the ten weeks feeding trial, 6 birds were randomly selected per treatment group and blood samples were collected through the wing vein from the overnight fasted birds using a hypodermic needle with a syringe. The blood samples from each bird was drained into two different carefully labeled bottles for haematological and serum biochemical analyses. Blood sample for haematological determinations were collected into disposable specimen test tubes containing ethylene diamine tetracetic acid (EDTA) as anticoagulant. Packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and haemoglobin (Hb) were determined by using improved Neubuar's haematocytometer after dilution and cyanometamoglobin as described by Dacie and Lewis (1991). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were determined by the method of Hyduke (1995). Serum biochemical traits such as total protein, albumin, creatinine and glucose, were determined by the method of Hyduke (1995), while the globulin was estimated by the subtraction of albumin value from serum total protein value (Dacie and Lewis, 1991). Total cholesterol (TC), triglycerides and high density lipoprotein (HDL) were determined by the methods described by Young, (2001) and Young et al. (1975). Low density lipoprotein (LDL) and very low density lipoprotein (vLDL) were estimated using the equations below as expressed by Friedewald, et al. (1972).

$$vLDL = \frac{Triglycerides}{5}$$

$$LDL = Total\ cholesterol - (HDL + vLDL)$$

Table 1: Nutrient composition of maize soybean basal layer diets

Nutrients	Percentage (%)
Maize	55.00
Soyabean meal	21.50
Fish meal	2.00
Wheat offal	10.70
Bone meal	7.00
Limestone	3.00
*Premix (Vit. & Min.)	0.30
Lysine	0.10
Methionine	0.10
Salt	0.30
Total	100.00
Calculated Analyses	
Crude protein (%)	17.26
Metabolizable energy (M.E Kcal/kg)	2606

* Vitamin-Mineral premix (Bio-mix) provided per kg include the following: Vitamin A 500 IU; Vitamin D3, 888,000 iu; Vitamin E, 12,200mg; Vitamin K3 12,000mg; Vitamin B1, 100mg; B2, 200mg; B6, 1500mg; Nacin, 1200mg; Pathothenic acid, 2000mg; Biotic, 100mg Vitamin B12, 3000mg; folic acid, 1500mg; Chlorine chloride, 60,000mg; Maganese, 10,00mg, Iron, 1500mg; Zinc, 800mg; Copper, 400mg; Iodine, 80mg; cobalt, 40mg; Selenium, 8000mg.

Statistical Analysis

Data obtained were subjected to a one way analysis of variance (ANOVA) and treatment means that significantly differed were compared using the Duncan's Multiple Range Test (DMRT) as outlined by Steel and Torrie (1990) using the SPSS (2014) IBM version 20.

III. RESULTS AND DISCUSSION

Results on haematological indices (Table 2) revealed that all the parameters assessed were significantly ($P<0.05$) affected by the treatments. PCV values were significantly ($P<0.05$) betterin layers maintained on T_4, T_3, T_1 and T_2 than those on T_5. However, values obtained in T_5 andT_2 were not statistically different from one another. The values recorded for PCV rangedfrom 32.00- 45.00% and were higher than 28.17-33.67% obtained for broilers treated withethanolicextract of *Chrysophyllum albidum* by Arogbodo et al.(2020) but fell within thereferencestandards of 32-45% reported by Nanbol et al.(2016). RBC values of broiler chickens ranged from 3.33 $x10^3$/ul in T_5 to 4.36 $x10^3$/ul in the controlgroup and were slightly higher than 3.12-3.57 $x10^3$/ul recorded by Makanjuola et al.(2014) who fed broiler chickens fed *Moringaoleifera* leaf meal but comparable to 3.06-3.96 $x10^3$/ul obtainedby Olumide and Odunowo (2019) for broiler chickens raised on diets supplemented with garlic.The WBC of broiler chickens were significantly ($P<0.05$) reduced by the MOLM and SOLMblend from 18.35$x10^6$/ul in the control group to 9.45 $x10^6$/ul in T_3 (1.0% MOLM + 1.5% SOLM)but the value in T_3 was similar that of T_4. Hb and MCV values were equally reducedsignificantly ($P<0.05$) by the MOLM

and SOLM mixture from 18.35g/dl and 13.40fl in thecontrol group to 10.25g/dl and 10.31fl respectively in T_5 group. MCH values were Significantly($P<0.05$) decreased by the mixture except for T_3 value (37.32pg) that compared favourably withthat of the control group (38.85pg).

Table 2: Haematological indices of laying hens as affected by the treatments.

Indices	Composite Mixture (MOLM + SOLM)					SEM±	Probability
	Control	0.5%+2.0%	1.0%+1.5%	1.5%+1.0%	2.0%+0.5%		
	Treatments (T)						
	1	2	3	4	5		
PCV (%)	44.83^{a}	39.00^{ab}	45.00^{a}	45.00^{a}	32.50^{b}	3.41	.000
RBC($x10^3$/µl)	4.36^{a}	3.37^{b}	4.26^{a}	4.28^{a}	3.33^{b}	0.34	.810
WBC($x10^6$/µl)	18.35^{a}	11.50^{d}	9.45^{c}	12.65^{c}	13.25^{b}	0.08	.000
Hb(g/dl)	18.35^{a}	11.25^{c}	15.26^{b}	15.25^{b}	10.25^{d}	0.13	.009
MCV(fl)	13.40^{a}	10.34^{c}	11.34^{b}	11.29^{b}	10.31^{c}	0.10	.006
MCH(pg)	38.85^{a}	33.95^{b}	37.32^{ab}	36.72^{b}	32.59^{d}	1.28	.722
MCHC (g/dl)	32.50^{d}	35.03^{c}	37.32^{a}	36.72^{b}	32.57^{d}	0.08	.579
Heterophil(%)	21.00^{d}	30.50^{b}	27.50^{c}	34.00^{a}	18.00^{e}	0.55	.879
LLymphocytes (%)	76.00^{a}	66.50b^{c}	70.00^{b}	62.50^{c}	75.00^{a}	1.45	.069

abcde: Means in the same row with different superscript differed significantly($P<0.05$).

The least value (32.59pg) of MCH was recorded in birds thathad T_5 andthe values ranged between 32.59 in T_5 and38.85pg in the controlgroup. These valueswere atpar with that of Bounous and Stedman(2000) whoreported 23-47pg foravian species.MCHCvalues of broiler chickens weresignificantly($P<0.05$) increased by the MOLM andSOLM blendexcept for T_5 (32.57g/dl) that was similar to that of the control group (32.50g/dl).The MCHCvalues in this study rangedfrom 32.50 to 37.32g/dl and these coincided with theranges of29.29±0.17-36.86±0.25g/dl and36.9-39.6g/dl obtained by Nihad et al.(2017)

andSugiharto etal.(2018) respectively for broiler chickens. The variation in the values of MCV, MCH andMCHC may be due to defense reaction against the MOLM and SOLM mixture owingto the stimulation of erythropoiesis (Esonu et al.,2006). Heterophil values were significantly($P<0.05$) different from one another with a range of 18.00-34.00%. The increase in the heterophil values due to the supplementation of the MOLM and SOLM mixture indicated the severity of the immune response of the birds to the test treatments. This observation underpinned those ofEsonu et al.(2006), Bonsu et al.(2012) and Nayaka et al. (2013) that neem leaf meal has thepotential to increase immunity of birds against protection. Lymphocytes values were significant($P<0.05$) and comparable to one another across the treatments with a range of 62.50% in T_4 to76.00% in the control group (T_1).An increase in lymphocyte counts is in response to viral,bacterial and parasitic infections of the animal body (Coles, 1986). Besides, they use the blood totravel round the body but can wonder freely in some tissues using the lymphatic channels.However, the values of heterophils and lymphocytes fell within the normal range by Mitruka andRawnsley (1977) suggestive of the fact that MOLM and SOLM mixture had no negative effecton the haematology of the birds. However, some of the mixtures decreased the values whichsuggest the need for caution in using them.

The serum chemistry analyses revealed that significant ($P<0.05$) variations existed in the values of total serum protein, albumin, globulins and glucose but creatinine values were not affected by the treatments (Table 3). Birds on T_2 had the highest value of 74.10g/dl total protein and least in those that had T_4 (68.10g/dl) but comparable values of 71.10 and 70.19g/dl were recorded in birds on the control group (T_1) and T_4 respectively. Total serum protein values ranged between 68.10 and 74.10g/dl and were slightly higher than 57.33±2.20 – 63.67±0.33g/L recorded by Arogbodo et al. (2020) for broiler chickens but disagreed with the range of 40-65g/L recommended for broiler chickens by Nanbol et al. (2016). The marked difference in the range of values in this study with the range of 4.55-5.13g/L obtained by Adetoro et al.(2017) for pullets maybe due to the wide disparity in the ages of the birds. Albumin values were significantly ($P<0.05$) higher in laying hens that took diets supplemented with MOLM and SOLM mixture compared to those on the control group. The values ranged between 38.70 and 42,55g/dl and were higher than22.67±3.67-37.33±1.20g/L for broiler chickens (Arogbodo et al., 2020). Globulin values also varied significantly ($P<0.05$) across the treatment groups with a range of 27.64-31.45g/dl which coincided with 23.67±3.28-32.67±2.91g/L obtained by Arogbodo et al. (2020). However, the values recorded for serum total protein, albumin and globulin were in line with the recommendations of Nanbol et

al.(2016) and Mitruka and Rawnsley (1977) for chickens and this points to the fact that the birds were of good health condition. This is because total protein and albumin is a measure of the biosynthetic function of the liver as it is the primary site for the synthesis of most plasma proteins (Hofferberg and Block, 1996). The serum creatinine of the laying hens were not significantly (P>0.05) affected by the supplementation of MOLM and SOLM mixture but higher numerical value was recorded in layers on the control group compared to those on other treatment groups. The values ranged from 0.55µmol/L in T_4 to 0.70µmol/L in the control group and were within the normal range for poultry birds recommended by Mitruka and Rawnsley (1977) and Ross et al.(1978). The non significant variation in the values recorded in the present study implies the protein quality of the diets as the value of creatinine is an indication of the protein quality (Eggum, 1970). It also portends the fact that the supplementation of MOLM and SOLM mixture had no adverse effect on the muscle wastage of the birds because higher serum creatinine indicates poor utilization of nutrients due to muscle wastage (Eggum, 1970). Glucose values of laying hens were reduced by the supplementation of MOLM and SOLM blend compared to that of the control group with a range of 245.40-179.55mmol/L and this underpinned the reports of Shalaby et al.(2006) and Onunkwo et al.(2019) who observed reduction in the serum glucose of broiler chickens due to galic powder supplementation.

Table 3: Serum biochemical indices of laying hens as affected by the treatments.

Indices	Composite Mixture (MOLM + SOLM)					SEM±	Probability
	Control	0.5%+2.0%	1.0%+1.5%	1.5%+1.0%	2.0%+0.5%		
	Treatments (T)						
	1	2	3	4	5		
Total protein (g/dl)	71.10^{c}	74.10^{a}	73.10^{b}	68.10^{e}	70.19^{d}	0.09	.000
Albumin (g/dl)	41.70^{b}	42.65^{a}	42.50^{a}	38.70^{c}	42.55^{a}	0.09	.000
Globulin (g/dl)	29.40^{c}	31.45^{a}	30.60^{b}	29.40^{c}	27.64^{d}	0.09	.000
Creatinine (µmol/L)	0.70	0.60	0.60	0.55	0.65	0.06	.506
Glucose (mmol/L)	245.40^{a}	230.45^{b}	194.65^{d}	179.50^{e}	200.40^{c}	0.06	.000

abcde: Means in the same row with varying superscripts differ (P<0.05)

From Table 4, it could be noticed that total cholesterol, triglyerides, low density lipoprotein (LDL) and very low density lipoprotein (vLDL) values of eggs laid by the hens were reduced significantly (P<0.05) by the supplementation of MOLM and SOLM mixture compared to the value obtained in birds on the control group. On the contrary, the high density lipoprotein (HDL) in eggs of birds on the control group was lower than those on other treatment groups. Total cholesterol values of eggs laid by the birds were reduced from 61.40mg/dl in the control group to 53.80mg/dl in T_5. This observation lend support from the findings of Chowdhary et al.(2002) who observed a decrease in serum and egg yolk cholesterol concentration of laying hens fed garlic paste and those of Chand et al.(2007) and Metwally (2009) who reported the cholesterol lowering property of *Berberis lyceum* and garlic respectively. The reduction in total cholesterol values in eggs of laying hens fed diets supplemented with MOLM and SOLM blend may be ascribed to the hypocholesterolemic agent or anti- oxidant compound such as flavanoid due to the formation of mevalonic acid from acetylCo_A which inhibit the activity of 3-methyl-3-hydroxyglutary Co_A (HMG-Co_A) reductase enzyme which convert acetylCo_A to mevalonic acid and reduce cholesterol (Ghasi et al., 2000). Triglycerides concentration of eggs significantly (P<0.05) decreased from 178.30mg/dl in the control group (T_1) to 159.45mg/dl in T_5. The reduction in the values of triglycerides due to the supplementation of MOLM and SOLM mixture may be due to the presence of alkaloids in the mixture (Chen et al., 2003; Brusq et al., 2006). Results of the present study is in concordance with those of Aghazadeh et al.(2011) and Rafiee et al.(2013) who reported reduction in the level of triglycerides of broiler chickens fed thyme+mint and ginger+thyme mixtures respectively. The use of MOLM and SOLM blend as supplement to the diets of the birds significantly (P<0.05) increased the HDL level of eggs from 10.65mg/dl in the control group to 20.45mg/dl in 2.0%MOLM + 0.5%SOLM blend. This is at par with the report of Abdul et al.(2012) and Awodola-Peters & Yahaya (2017) who observed a significant increase in the HDL level of broiler chickens administered different herbal infusions and graded levels of roselle calyx respectively. The present results also conformed with the opinion of Nihad et al.(2017) that the supplementation of *Moringa oleifera* leaf powder significantly increased the HDL of broiler chickens. HDL also known as "high quality" cholesterol carries the cholesterol from the blood vessels and body tissues to the liver for reutilization or excretion from the body. It also helps to keep blood vessels dilated thereby enhancing blood flow, reduces blood vessel

injury through its anti-oxidants and anti-inflammatory functions. Furthermore, increased level of HDL had been adjudged to reduce the risk of heart diseases, stroke and health problems (Abdul et al., 2012). This implies that the consumption of eggs laid by hens on 2.0%MOLM+.05%SOLM supplement will lead to high intake of dietary HDL since the concentration was markedly higher than those of other treatment groups. LDL and vLDL values of laying hens due to the supplementation of MOLM and SOLM mixture decreased from 14.82mg/dl and 35.66mg/dl in the control group to 8.19mg/dl and 31.89mg/dl in T_5. The reduction of LDL and vLDL values of laying hens by the supplementation of MOLM and SOLM is in line with that of Abousekken (2015) who observed a reduction in LDL and vLDL values of broiler chickens fed low protein diets supplemented with MOLM or its extracts. Similar observations were made by Attia et al. (2016), AbdEl-Hady et al. (2017) and Nihad et al.(2017) who fed broiler chickens with plant extract blend, black mulberry fruit juice and MOLM respectively. AboEl-Maaty et al. (2018) also observed significant improvement on the growth performance and liver histology of broiler chickens fed some leaves extract of medicinal plants as anti-oxidants. The present results therefore connote a reduction in the risk of heart disease from the consumption of eggs laid by birds on MOLM and SOLM blend.This is because LDL also known as "bad cholesterol" is the major cholesterol carrier in the blood. If too much LDL cholesterol circulates in the blood, it can slowly build up in the walls of arteries feeding the heart and the brain. In addition, it can with other substances form a plaque, a thick and hard deposit that can clog the arteries. Therefore, a high level of LDL cholesterol in the blood reflects a high risk of cardiovascular disease. The antherogenic index in this study which is the ratio of LDL to HDL were lower in birds maintained on MOLM and SOLM supplement compared to that of the control group. This suggest that the consumption of eggs laid by birds raised with diets supplemented with MOLM and SOLM mixture will reduce the risk of cardiovascular disease as this index is a strong marker in the prediction of cardiovascular disease in farm animals.

IV. CONCLUSION

The combination of MOLM and SOLM was beneficial to the health status of the laying birds as depicted by the haematological and serum biochemical indices. The MOLM and SOLM blend significantly increased the HDL and reduced the total cholesterol, triglyceride, LDL and vLDL concentration in eggs laid by the birds. Therefore, a mixture of 2.0%MOLM and 0.5%SOLM could be included in the diets of laying hens to promote good health and improve the lipid profile of eggs as well as an alternative to antibiotics.

Table 4: Egg lipid profile of layers as affected by treatments

	Composite Mixture (MOLM + SOLM)						
	Control	0.5% + 2.0%	1.0%+1.5%	1.5%+1.0%	2.0%+0.5%		
	Treatments (T)						
Indices	1	2	3	4	5	SEM±	Probability
Total cholesterol (mg/dl)	61.40^{a}	60.50^{b}	58.45^{d}	59.35^{c}	53.80^{e}	0.05	.089
Triglyceride (mg/dl)	178.30^{a}	170.40^{b}	168.25^{c}	166.50^{d}	159.45^{e}	0.09	.075
HDL (mg/dl)	10.65^{e}	12.55^{d}	13.70^{c}	15.30^{b}	20.45^{a}	0.09	.054
LDL (md/dl)	14.82^{a}	9.55^{c}	9.09^{d}	10.78^{b}	8.19^{e}	0.02	.000
vLDL (mg/dl)	35.66^{a}	34.11^{b}	33.68^{c}	33.33^{d}	31.81^{e}	0.02	.000
Antherogenic index	1.39	0.76	0.66	0.71	0.40	-	-

abcde: Means in the same row with varying superscripts differ (P<0.05)

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