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Maximizing Productivity of Lohmann Chickens by Feeding Diets Inclusion Different Levels of *Moringa oleifera* Leaf Powder as a Safe Feed Additive

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ABSTRACT

The purpose of the present study was to investigate the effect of inclusion graded levels of *Moringa oleifera* leaf powder (MOLP) as a safe feed additive on productive performance, some blood parameters, egg quality traits and egg cholesterol contents of Lohamnn chicken. A total number of 150 hens, aging 20 weeks were randomly allocated in a completely randomized design to 5 dietary treatments, in each three replicates (n=10) and their initial mean body weight ranged from 1501.0 to 1530.0 g (±7.01), where each 2 birds were placed in one cage. The control group was fed a corn-soybean meal based diet and the other experimental groups were fed on control diet supplemented with 0.5%, 1.0%, 1.5% and 2.0% MOLP. The results revealed that laying performance statistically (P≤0.05) improved due to the addition of MOLP as compared to the control group. Furthermore, FCR recorded the best values for groups fed MOLP, which displayed relatively high performance. However, inclusion of MOLP in diets improved both egg shell thickness and yolk color. In addition, egg cholesterol contents significantly (P≤0.05) decreased due to increasing levels of MOLP in diets. Obliviously, health status as judged by measuring some blood constituents, showed decreases (P≤0.05) of lipid profile and Corticosterone concentration, while level of HDL and protein profile significantly (P≤0.05) increased due to the addition of MOLP in the diets. Clearly, it can be recommended that the incorporating of MOLP in Lohmann chickens diets could enhance their productive performance and improve physiological status.

Keywords: Lohmann chicken, *Moringa oleifera* leaf powder, laying performance

INTRODUCTION

It is well known that global poultry meat and egg production as well as trade with poultry products have shown a remarkable dynamic during the last 35 years (Windhorst, 2006). Therefore, the poultry industry is one of the fastest growing animal industries globally, where poultry production plays a major role in bridging the protein gap in developing countries where average daily consumption is far below recommended standards (Onyimonyi *et al.,* 2009). The chicken egg is one of the most valuable and most perfect foodstuffs of animal origin, where eggs are an important source of nutrients, it contains proteins, lipids, vitamins, and minerals, which ensure the development of the living organism and provide a defense system against infection (Rzedzicki, and Stępień-pyśniak, 2009). In poultry, herbs and spices are not only appetite and digestion stimulants, but also impact other physiological functions, help to sustain good health and welfare, and improve their performance (Frankic *et al.,* 2009). However, World Health Organization encourages the use of medicinal herbs and plants to minimize the use of chemicals through the global trend to go back to nature. Attempts to use natural materials such as medicinal plants could be widely accepted as feed additives to enhance efficiency of feed utilization and productive performance (Levic *et al.,* 2008). The early studies of Francois (2006) found that medicinal herbs are desirable for stimulating digestion, particularly affecting bile secretion and activity of pancreatic enzymes. On the other hand, the harmful effect of high ambient temperature on egg production has been well studied. Poultry production sectors in developing countries are facing some problems, where a temperature above 30°C represents a heat stress condition in poultry and considered one of the most common stress that affect the production criteria. Therefore, the researchers have tried to minimize the effect of heat stress by changing the environment and diets of laying hens (Çiftçi *et al.,* 2005). Indeed, poultry production sectors in developing countries are facing some problems, one of which is increase antibiotic resistant pathogens due to unwise and excessive use of antibiotics. Researchers are therefore looking for cheap, available, and safe alternative sources natural products. Recently, there has been great interest in the use of Moringa as natural antioxidants, has antimicrobial activity, which plays a significant role in nutritional, medicinal and therapeutic values (Al-Kharusi *et al,* 2009; Siddhuraju and Becker, 2003). Therefore, Moringa leaves are rich in biologically active such as polyphenols, tannins, anthocyanin, glycosides and thiocarbamates, which remove free radicals, activate antioxidant enzymes and inhibit oxidases (Luqmans *et al.,* 2012). Recently, Lu *et al.* (2016) showed that dietary supplementation with 5% Moringa leaves could improve yolk colour value and protein absorption without adverse effects on laying performance and egg quality of chickens. For this aim, the main purpose of this study was to investigate the effect of incorporating MOLP at different levels on performance, some blood parameters, egg quality traits and egg cholesterol content of Lohmann laying hen.

MATERIALS AND METHODS

Site and the aim of study: The present study was conducted at Siwa Research Station, belonging to El-Marsa Matrouh Desert Research Center, Ministry of Agriculture and Land Reclamation, Egypt, between the periods of March 2015 to June, 2015. The main purpose of this study was to asses the effect of incorporating *Moringa oleifera* leaf powder (MOLP) at different levels on performance, some blood parameters, egg quality traits as well as egg cholesterol content of Lohman laying hen.

Preparation of MOLP, experimental diets and chemical analysis: One batch (100 kg) of fresh matured Moringa leaves was obtained from National Research Center, (Moringa society), and dried naturally in a sunny then the leaves were ground using a mixer and stored in bags at ambient temperature (27–30oC) before being mixed into diets. The proximate chemical compositions, amino acid, mineral, vitamin contents and bio-active components of MOLP are shown in Tables 2, 3, 4 and 5 respectively. All diets were isocaloric and isonitrogenous and formulated using yellow maize and soyabean meal as main ingredients

according to nutrient specifications recommended by (NRC 1994). The experimental groups were fed the control diet supplemented with 0.5, 1.0, 1.5 and 2.0% MOLP as shown in Table 1. The proximate chemical analyses for experimental diets and MOLP were determined according to the Association of Official Analytical Chemists (AOAC, 1984).

Table 1. Compositions and chemical analysis of the experimental diets.

Ingredients	Dietary treatments				
	Control (T1)	T2 (0.5%)	T3 (1.0%)	T4 (1.5%)	T5 (2.0%)
Yellow corn (8.5%)	62.40	62.00	62.00	61.50	61.50
Soybean meal (44 %)	24.00	23.90	23.50	23.50	23.00
Corn gluten meal (60%)	3.00	3.00	3.00	3.00	3.00
Moringa oleifera leaf	0.00	0.50	1.00	1.50	2.00
DL-Methionine	0.10	0.10	0.10	0.10	0.10
Di-calcium phosphate	1. 20	1. 20	1. 20	1. 20	1. 20
Premix*	0.30	0.30	0.30	0.30	0.30
Limestone	8.70	8.70	8.60	8.60	8.60
Sodium Chloride	0.30	0.30	0.30	0.30	0.30
Total (Kg)	100.00	100.00	100.00	100.00	100.00
Calculated analysis:					
Crude protein (%)	17.82	17.78	17.74	17.83	17.74
ME (Kcal / kg)**	2734	2731	2733	2726	2725
Ether extract (%)	2.64	2.71	2.79	2.85	2.94
Crude fiber (%)	3.09	3.17	3.34	3.33	3.39
Lysine (%)	0.84	0.84	0.82	0.83	0.82
Methionine (%)	0.56	0.55	0.55	0.56	0.57
Calcium (%)	3.73	3.73	3.74	3.73	3.72
Total phosphorus (%)	0.57	0.57	0.57	0.58	0.57
Av. phosphorus (%)	0.34	0.34	0.34	0.35	0.34
Chemical analysis:					
Crude protein (%)	17.77	17.75	17.72	17.81	17.71
Crude fiber (%)	2.883	2.877	2.886	2.884	2.879
Ether extract (%)	2.85	2.84	2.84	2.83	2.82
Calcium (%)	3.81	3.80	3.85	3.89	3.82
Av. phosphorus (%)	0.321	0.324	0.319	0.331	0.325

*** Pre-mix each 3 kg of vitamin and mixture contains: 10000000 IU Vit. A, 2000000 IU Vit.D3 10000 mg Vit.E, 1000 mg Vit.K, 1000 mg Vit.B1, 5000 mg Vit.B2, 1500 mg Vit.B6, 10 mg Vit.B12, 10000 mg Vit. pantothenic acid, 30000 mg Vit.Nicotinic acid, 1000 mg Vit.Folic acid, 50 mg Vit.Biotin, 100 mg cobalt, 4000 mg Copper sulphate, 300 mg Ca iodide, 30000 mg ferrous sulphate, 60000 mg Manganese oxide, 50000 mg Zinc oxide and 100 mg Sodium selenite.** ****ME=Metabolizable energy**

Experimental birds and their management : A total number of 150 hens, aging 20 weeks were randomly allocated in a completely randomized design to 5 equal dietary treatments , in each three replicates (n=10) and initial mean body weight ranged from 1501.0 to 1530.0 g (±7.01), where each 2 birds were placed in one cage. The hens were housed in pyramid battery cages with dimension of 40 x 50 x 50 cm. The lighting schedule consisted of 17 h light and 8 h darkness provided by incandescent bulb lamps of 6-8 watts /m2. Before allocation of birds, the house was carefully cleaned and disinfected. The hens were vaccinated against Marek and Newcastle diseases. Also, programs of multivitamin addition and anti-worms drug were applied along the experimental period. Similar management conditions were maintained for all groups according to the management guide of Lohmann chickens (Lohmann, 2010). Temperatures and humidity were daily recorded within the experimental house using a standard thermometer and a hygrometer. The experiment was carried out from the beginning of March 2015 to June, 2015.

Experimental measurements

Productive trait: The hens were weighed at the start and at the end of the experiment and body weight change was calculated as the difference between the final and initial weight. Birds were provided with diet once daily in the morning, where the amount provided in each feeder was weighed an allowance of about 20-25% above the expected daily requirements presented in guideline. The refusals were weighed the next day, just before provision of another diet. All groups were inspected daily, to detect any problem. Egg production was collected every day in the morning and evening per replicate and recorded separately. Egg weight was determined by weighing all egg for each replicate alone. Feed conversion ratio (FCR) was obtained on a weekly basis and it was measured by dividing the feed intake by weight of eggs produced for each replicates as follows:

FCR = feed intake (g) / egg mass (g)

However, hen- day egg production rate was determined according to Hunton (1995) equation as follows: % hen- day egg production = total number of eggs produced /total number of hens present on that day x100 Egg quality traits and cholesterol contents determination: Egg quality traits were done on the same day once all samples were collected. Fifty fresh eggs were used to measure egg quality traits and yolk cholesterol contents per period. Freshly laid eggs were individually weighed on an electric balance, accurate to 0.01 g. All eggs were individually broken on a flat surface and different components were carefully separated and weighed. Shell thickness was determined, where the mean value of measurements at 3 locations on the egg (air cell, equator and sharp end) by using a dial gauge micrometer according to Yannakopoulos and Tserveeni-Gousi (1986). The yolks were separated using a Teflon spoon. Before estimation the yolk weight, the chalaza was removed with a spatula. Yolk color was scored using a colorimetric fan (Roch). Percentages of egg components (shell, yolk and albumin relative to total egg weight were determined according to Stadelman and Cotterill (1995).

Egg shape index was measured according to the following formula:

Shape index= (short axis / long axis) x100 (Yannakopoulos and Tserveeni-Gousi, 1986).

Yolk height was measured by a tripod micrometer (Mitutoyo, 0.01mm, Kawasaki, Japan). Yolk's diameter was measured and the yolk index was calculated according to the following formula:

Yolk index= (yolk height / yolk diameter) x 100 (Kirikci *et al.*, 2007).

Haugh units were calculated according to (Cotta, 1997):

HU = 100 Log (h – 1.7 w + 7.6), in which HU = Haugh unit, h = albumen height (mm) and w = egg weight (g).

For determination of cholesterol, the yolks were taken for lipid extraction, one gram of yolk was placed into a centrifuge tube, homogenized with 15 ml of polar solvents chloroform: methanol mixture, 2:1 (v/v), vortexed, filtered and evaporated according to Folch et al. (1957), as modified by Washburn and Nix (1974).

Blood constituents: Approximately 3 mL of blood were obtained from the jugular veins at the end of experiment. Plasma was separated by centrifuging the tube contents at 3000 rpm for 15 minutes. Samples were stored in a deep freezer at -20oC until the time of chemical analyses. Biochemical constituents including total protein (Gornal *et al.,* 1949), albumin (Doumas *et al.,* 1971), total lipid (Zollner and Kirsch, 1962), total cholesterol, (Allain *et al.,* 1974), triglycerides (Fossati and Prencipe 1982), LDL and VLDL (Sawle *et al.,* 2002), HDL (Lopez -Virella *et al.,* 1977), ALT and AST (Retiman and Frankel, 1957), were done by using a standard commercial diagnostic kits. The globulins levels was obtained by subtracting the values of albumin from the corresponding values of total protein. Also albumin/ globulin (A/G) ratio were obtained by dividing the values of albumin on that of globulins. Levels of Plasma triiodthyronine (T3) and thyroxin (T4) were determined according to Schalm *et al.* (1975) with commercial human enzyme immunoassay kit. Also, Corticosterone levels were determined by radioimmunoassay(RIA) procedure according to Simensen *et al.* (1978).

Detection of volatile substances: Three grams of the samples were subjected to water distillation (500 ml water) using Clevenger's apparatus. The distillation was continued for 3 hr after boiling. The volatile substances were isolated and dried over anhydrous Na2SO4 (Guenther, 1953). Bio-active components including carbohydrates and/or glycosides (Lewis and Smith, 1967), tannins (Shellard, 1957), flavonoids (Geissman, 1961), Saponins (Shellard, 1957), Triterpenes (Hanson, 1972), alkaloids and/or nitrogenous bases (Farnsworth *et al.,* 1964), and coumarins (Feigl, 1960) were determined.

Statistical design: The experiment was arranged in a complete randomized design. Then one-way analysis of variance was employed using the SPSS procedure (SPSS for Windows Release 16, SPSS Inc. 2005). The differences among groups were evaluated by Duncan's (Duncan's 1955) multiple comparison tests. Differences were considered statistically significant at ($P \leq 0.05$). Statistical analysis of traits presented as percentages was carried out for arcsine values of their estimates. Data are expressed as mean and pooled SEM, where the statistical model was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where, Y_{ij} = individual measurement, μ = overall mean effect, T_i = effect of dietary MOLP, e_{ij} = error term

RESULTS

Proximate chemical compositions of MOLP and bioactive components: The results of the proximate chemical compositions, amino acids, minerals, vitamins and bio-active components are shown in Tables 2, 3, 4 and 5. The obtained results indicated that MOLP is rich in macronutrients, where the values of dry matter, moisture, organic matter, crude protein, gross energy, ether extract, crude fiber, ash and nitrogen free extract were found to be 91.53, 8.57, 88.03, 28.11, 4216, 4.71, 9.34,11.97 and 37.4 % respectively. Obviously, 17 amino acids were identified, where both essential and non-essential amino acid are present in MOLP. Surely, amino acid contents and their availability are major important in the nutritional assessment. Interestingly, MOLP has higher contents of amino acid, especially Leucine (2.701 mg/g protein), and tyrosine (3.86 mg/g protein), while threonine and isolucine displayed the lowest contents (0.134 v., 1.635 mg/g protein). The highest values of nonessential amino acid were glutamic acid, aspartic acid and alanine (3.380, 2.650, and 2.601 mg/g protein), respectively. Therefore, MOLP contains considerable levels of lysine and methionine (0.337 vs., 1.577 mg/g protein). Clearly, MOLP seems to be a good source of both essential and non-essential amino acids. However, the mineral analysis of MOLP indicated that the macro-elements present were calcium (3.55), phosphorous (0.3), potassium (1.5), magnesium (0.5), sulphur (0.63) and sodium (0.164 g / kg). The micro-elements were zinc (3.3), copper (0.8), iron (49), manganese (8.6), selenium (36.3) and boron (4.99 mg / kg), respectively. It was noted that MOLP has considerable contents of macro and micro-elements, especially iron and selenium compared with other elements. On the other hand, the MOLP also contained high amounts of both vitamin E (113mg /kg) vs., B2 (20.5 mg /kg), followed by vitamin C (17.5), A-B carotene (16.25), vitamin B3 (8.2), and vitamin B1 (2.6 mg /kg) respectively, considered excellent source of many vitamins and minerals, that has been known for its health benefits. Concerning the bioactive components present in MOLP, the analysis indicated that MOLP contained relatively higher levels of glycosides, tannins, and triterpenes than steam volatile substance, flavonoids, saponins and alkaloids. While, MOLP has no contains coumarins.

Table 2. Proximate chemical composition of MOLP (% of dry matter basis)

Items	Nutrient contents
Macronutrients	
Dry matter (%)	91.53
Moisture (%)	8.57
Organic matter (%)	88.03
Crude protein (%)	28.11
Gross energy (kcal/kg)	4216
Ether extract (%)	4.71
Crude fiber (%)	9.34
Ash (%)	11.97
NFE (%)	37.4

MOLP= Moringa oleifera leaf powder

Table 3. Amino acid compositions of MOLP

Amino acids	mg/g protein
Essential	
Lysine	1.577
Leucine	2.701
Valine	1.676
Histidin	1.472
Methionine	0.337
Tryptophan	0.442
Threonine	0.134
Tyrosine	3.860
Isolucine	1.635
Phenylalanine	2.330
Argnine	1.90
Nonessential	
Aspartic acid	2.650
Glutamic acid	3.380
Alanine	2.601
Cystine	1.480
Proline	1.230
Glycine	1.830

Table 4. Mineral and vitamin compositions of MOLP

Items	Nutrient contents
Macro elements (g/kg)	
Calcium	3.55
Phosphorous	0.3
Potassium	1.5
Magnesium	0.5
Sulphur	0.63
Sodium	0.164
Micro elements (g/kg)	
Zinc	3.3
Copper	0.8
Iron	49
Manganese	8.6
Selenium	36.3
Boron	4.99
Vitamin (mg /kg)	
Vitamin (A-B) carotene	16.25
Vitamin (B1) Thiamin	2.6
Vitamins (B2) Riboflavin	20.5
Vitamins (B3) Nicotinic acid	8.2
Vitamins (C) Ascorbic acid	17.5
Vitamins (E)A Tocopherols	113

Table 5. Preliminary phytochemical screening of MOLP

Test	Result
Steam volatile substance	+
Carbohydrates and/or glycosides	++
Flavonoids	+
Tannins	++
Saponins	+
Sterol and/or Triterpenes	++
Coumarins	-
Alkaloids	+

(++), (+), and (-): refer to high, low and absent amount of certain compounds, respectively.

Productive performance: Results of productive performance as affected by MOLP addition are outlined in Table 6. From the present results, it is observed that the initial body weigh was similar among the experimental groups and ranged between 1501 to 1530 (±7.01), reflecting insignificant differences at start of experiment. While, at the end of laying period the analysis of variance indicated that birds fed diet included 2% MOLP recorded significantly ($P \leq 0.05$) higher final body weight, than those fed 1.5, 1.0, 0.5% MOLP or control group respectively. However, the change in body weight recorded higher positive values for birds fed 2.0% and 1.5% MOLP, followed by those fed 1.0, 0.5% and control one. Daily egg production recorded during 22-25 weeks indicated the absence of significant differences among the experimental groups. While, egg production recorded during either 26-29 or 30-33 weeks showed that birds fed 1.0 % MOLP exhibited the highest values compared with other MLOP groups or control one. Concerning egg weight, birds fed 1.0, 0.5 and 1.5% MOLP recorded significantly higher ($P \leq 0.05$) values compared with those fed 2% MOLP or control group during 22-25weeks. However, during 26-29 weeks, egg weight recorded the highest values for birds fed 1.5, 1.0, 0.5% MOLP and control compared with those fed 2 % MOLP. During 30-33 weeks birds fed 1.0 % MOLP recorded the highest egg weight compared with other dietary MOLP groups or control. The values of egg mass observed during 22-25 weeks showed insignificant differences among the experimental groups, whereas during the period 26-29 weeks egg mass recorded significantly higher values for birds fed diets included 1.0 and 1.5% MOLP compared with other MOLP groups or control one. However, egg mass produced during 30-33 weeks indicated that the highest means were observed for birds fed 1% MOLP, followed by those fed 1.5%, control, 0.5% and 2% MOLP. While, the values of feed intake significantly ($P \leq 0.05$) decreased due to inclusion of MOLP in the diets for the different periods of experiment. Moreover, FCR calculated during 22-25 weeks was insignificantly affected due to addition MOLP. While, during 26-29 and 30-33 weeks, the analysis of variance indicated that there were improvements in FCR, where the best values were recorded for birds fed MOLP compared with control one. It is interesting to note that the addition of MOLP led to an enhancement of most productive traits.

Blood constituents: Table 7 shows some blood constituents as affected by inclusion different levels of MOLP in laying hen diets. Results indicated that protein profile including total protein, albumin and globulin significantly ($P \leq 0.05$) increased due to increasing level of MOLP in the diets. In opposite trend, the values of A/G ratio significantly decreased due to addition MOLP. Concerning, the lipid profile including total lipids, triglycerides, cholesterol LDL and VLDL, the analysis of variance indicated that there were significant ($P \leq 0.05$) decreases for these components, as MOLP increased in the diets. In contrast HDL significantly ($P \leq 0.05$) increased when MOLP increased in the diets. Concerning ALT and AST enzymes there were insignificant differences among the experimental groups. Concerning the hormone assays, data indicated that T3 and T4 significantly increased, when MOLP level increased in the diets. While, Corticosterone concentration markedly ($P \leq 0.05$) decreased by increasing level of MOLP in the diets.

Egg quality traits and yolk cholesterol content: Data of egg quality traits and egg cholesterol content of Lohmann chickens as affected by dietary level of MOLP are illustrated in Table 8. The data indicated that albumin weight percentage was not significantly different among the experimental groups during 22-25 weeks. While, during 26-29 weeks, the highest albumen percentage recorded for birds fed 2.0% MOLP compared with other dietary treatments. However, the albumen percentage recorded during 30-33 weeks indicated that the highest values was achieved for birds fed 1.5 and 2.0% MOLP, followed by those fed 1.0%, control and 0.5% MOLP, respectively. Yolk weight percentage observed during 22-25 weeks showed insignificant differences among the experimental groups. In contrast, the highest yolk weight

percentage observed for birds fed 1.0 % MOLP, compared with other treatments during 26-30 weeks. For 30-33 weeks the highest yolk percentage observed for birds fed either 0.5% MOLP or control one compared with other treatments. The shell weight percentage recorded during 22-25 weeks exhibited higher value for birds fed 1.0% MOLP than those fed either MLOP or control group. During the period 26-30 weeks the highest value observed for birds fed 0.5% MOLP compared with other treatments. While during 30-33 weeks shell weight percentage insignificantly affected due to addition of MOLP in the diets. The data of yolk index, birds fed 1.0 % MOLP had the highest during 22-26 weeks, while during the periods 26-30 and 30-343 weeks there were insignificant differences observed among the experimental groups. The result therefore indicated that inclusion of MOLP at different levels significantly improved either yolk color or shell thickness, where the highest values recorded for groups supplemented high level 2.0%, followed by those fed 1.5,1.0 and 0.5% compared with control group. Haugh units exhibited the highest values for birds fed 1.5% MOLP during 22-26 weeks compared with other treatments, while insignificant differences detected among the experimental groups for other periods. As presented in Table 8, the addition of MOLP in the diets of Lohmann chickens had beneficial effects for decreasing cholesterol contents of eggs produced thought the experiment. The analysis of variance indicated that cholesterol (mg/g yolk) contents significantly (P≤0.05) decreased for eggs resulted from birds fed MOLP compared with eggs produced from control group for all period of experiment.

Table 6. Productivity of Lohmann chicken as affected by inclusion different levels of MOLP in the diets at different periods of experiment (Means± SE*)

Parameters	Dietary treatments						
	T1 control	T2 (0.5%)	T3 (1.0 %)	T4 (1.5%)	T5 (2.0 %)	SEM	Sig.
BW (g)[1]:							
Initial	1508.3	1501.0	1530.0	1508.3	1506.7	7.01	NS
Final	1521.7 ^{b}	1558.3 ^{b}	1625.0^{a}	1655.0 ^{a}	1663.3 ^{a}	15.67	*
CBW[2]	13.4^{d}	57.3^{c}	95.0^{b}	146.7^{a}	156.6^{a}	15.88	
DEP (%) [3]:							
22-25 Weeks	59.72	60.42	62.50	61.81	59.67	0.82	NS
26-29 Weeks	79.27 ^{b}	80.84 ^{ab}	82.33 ^{a}	81.63 ^{ab}	80.36 ^{ab}	0.44	*
30-34 Weeks	83.66 ^{b}	83.72 ^{b}	86.21 ^{a}	83.99 ^{ab}	83.52 ^{b}	0.38	*
DEW (g)[4]:							
22-25 Weeks	54.20 ^{ab}	56.07 ^{a}	56.28 ^{a}	55.33 ^{a}	53.91 ^{b}	0.41	*
26-29 Weeks	61.06 ^{a}	61.21 ^{a}	61.08 ^{a}	62.00 ^{a}	59.52 ^{b}	0.25	*
30-34 Weeks	61.65 ^{ab}	61.55 ^{ab}	62.56 ^{a}	62.17 ^{ab}	60.91 ^{b}	0.21	*
DEM (g/birds) [5]:							
22-25 Weeks	975	1015	1052	1026	964	17.06	NS
26-29 Weeks	1447^{bc}	1484^{abc}	1508^{a}	1518^{a}	1435 ^{c}	10.41	*
30-34 Weeks	1547 ^{b}	1545 ^{b}	1618 ^{a}	1566 ^{b}	1526 ^{b}	10.28	*
DFI(g/bird)[6]:							
22-25 Weeks	94.3 ^{ab}	89.8 ^{b}	89.0 ^{b}	91.2 ^{b}	89. 9 ^{b}	0.94	*
26-29 Weeks	104.4 ^{a}	95. 6 ^{b}	100.0 ^{b}	99.5 ^{b}	96. 2 ^{b}	0.98	*
30-34 Weeks	112. 6 ^{a}	106.0 ^{ab}	104.9 ^{b}	100.5^{b}	105.2 ^{b}	1.30	*
FCR (g feed/ g egg) [7]:							
22-25 Weeks	2.90	2.65	2.53	2.66	2.80	0.06	NS
26-29 Weeks	2.16 ^{a}	1.93 ^{b}	1.99 ^{b}	1.96^{b}	2.01 ^{b}	0.02	*
30-34 Weeks	2.18 ^{a}	2.06 ^{b}	1.95 ^{b}	1.92 ^{b}	2.06 ^{b}	0.03	*

^{abc} Means with different superscripts within the same row are significantly different (P ≤ 0.05) *SE=Stander error of mean

1-BW=Body weight, 2- CBW= change in body weight, 3-DEP=hen –day egg production rate, 4- DEW=daily egg weight, 5- DEM=daily egg mass, 6-DFI=Daily feed intake, 7-FCR= feed conversion ratio

Table 7. Some blood parameters of Lohmann chicken as affected by inclusion different levels of MOLP in the diets at different periods of experiment (Means± SE)

Parameters	Dietary treatments						
	T1(control)	T2 (0.5 %)	T3 (1.0%)	T4 (1.50%)	T5 (2.0%)	SEM	Sig
TP (g/dl)[1]	6.51^{c}	7.22^{b}	7.58 ^{ab}	7.75 ^{ab}	7.83 ^{a}	0.14	*
TA (g/dl)[2]	3.79 ^{d}	4.10 ^{c}	4.27 ^{b}	4.35 ^{b}	4.41 ^{a}	0.06	*
TG (g/dl)[3]	2.72 ^{b}	3.12 ^{ab}	3.30 ^{ab}	3.40 ^{ab}	3.41 ^{a}	0.10	*
A/G ratio[4]	1.39 ^{a}	1.31^{ab}	1.29 ^{b}	1.27 ^{b}	1.29 ^{b}	0.20	*
TL (mg/dl)[5]	1029.10 ^{a}	920.30 ^{b}	929.87 ^{ab}	909.93 ^{b}	879.63 ^{b}	17.99	*
TRG (mg/dl)[6]	171.51 ^{a}	168.84 ^{ab}	165.85 ^{ab}	160.69 ^{ab}	157.98 ^{b}	1.89	*
CHOL (mg/dl)[7]	144.38 ^{a}	140.26 ^{ab}	139.28 ^{ab}	137.82 ^{ab}	134.49 ^{b}	1.33	*
LDL (mg/dl)[8]	123.06 ^{a}	113.90 ^{ab}	108.86 ^{ab}	104.55 ^{ab}	98.25 ^{b}	2.69	*
VLDL (mg/dl)[9]	34.30 ^{a}	33.77 ^{ab}	33.17 ^{ab}	32.14 ^{ab}	31.60 ^{b}	0.38	*
HDL (mg/dl)[10]	55.63 ^{b}	60.14 ^{ab}	63.59 ^{ab}	65.40 ^{ab}	67.83 ^{a}	1.55	*
AST (U/ml)[11]	49.95	49.25	46.87	46.38	44.81	1.02	NS
ALT(U/ml)[12]	37.31	33.03	31.66	28.66	28.28	1.74	NS
T3 (ng/ml)[13]	2.44 ^{c}	2.83 ^{b}	3.45 ^{a}	3.52 ^{a}	3.57 ^{a}	٠.١٢	*
T4 (ng/ml)[14]	12.7 ^{d}	13.38 ^{c}	14.03 ^{b}	14.69 ^{a}	14.75 ^{a}	٠.٢١	*
CS (n mol/L) [15]	1.79 ^{a}	1.68^{ab}	1.65^{ab}	1.58^{b}	1.53^{b}	0.03	*

^{abc} Means with different superscripts within the same row are significantly different (P ≤ 0.05)

1-TP= Total protein,2- TA=Total albumin, 3-TG=Total globulin, 4-A/G =albumin/ globulin ratio, 5- TL=Total lipids,6-TRG= triglyceride, 7-CHOL= cholesterol, 8-LDL=Low demerit lipoprotein, 9-VLDL=Very low density lipoprotein, 10- HDL=High density lipoprotein, 11- AST= Aspartate aminotransferase,12- ALT=Alanine aminotransferase, 13- T3=Triiodothyronine, 14-T4=Thyroxin, 15-CS=Corticosterone.

Table ^. Egg quality traits and cholesterol contents of Lohmann chickens as affected by inclusion different levels of MOLP in the diets at different periods of experiment (Means± SE).

Parameters	Dietary treatments						
	T1 (control)	T1 (05%)	T2 (1.0%)	T3 (1.5%)	T4 (2.0%)	SEM	Sig.
AW (%)[1]:							
22-26 Weeks	65.40	65.95	64.69	65.65	67.27	0.39	NS
26-30 Weeks	63.71 [b]	62.43 [b]	63.61 [b]	63.27 [b]	65.29 [a]	0.30	*
30-34 Weeks	64.20 [ab]	63.28 [b]	64.65 [ab]	65.70 [a]	65.61 [a]	0.34	*
YW (%)[2]:							
22-26 Weeks	22.05	21.00	21.80	21.97	21.65	0.26	NS
26-30 Weeks	23.80 [ab]	24.23 [a]	23.93 [ab]	23.70 [ab]	22.43 [b]	0.24	*
30-34 Weeks	23.61 [a]	23.82 [a]	22.31 [b]	22.45 [b]	21.67 [b]	0.25	*
SW (%)[3]:							
22-26 Weeks	12.56 [ab]	13.05 [ab]	13.51 [a]	12.38 [b]	11.08 [c]	0.25	*
26-30 Weeks	12.49 [bc]	13.34 [a]	12.47 [bc]	13.03 [ab]	12.28 [c]	0.13	*
30-34 Weeks	12.19	12.90	13.04	11.85	12.72	0.29	NS
YI[4]							
22-26 Weeks	46.66 [b]	48.39 [ab]	50.89 [a]	45.70 [b]	48.95 [ab]	0.65	*
26-30 Weeks	45.78	45.80	44.37	44.32	48.53	0.68	NS
30-34 Weeks	47.50	47.02	46.62	48.28	47.73	0.41	NS
YC[5]:							
22-26 Weeks	7.00 [c]	7.67 [c]	9.33 [b]	9.67 [ab]	12.00 [a]	0.49	*
26-30 Weeks	7.67 [c]	8.33 [c]	8.67 [b]	10.67 [ab]	12.00 [a]	0.45	*
30-34 Weeks	8.00 [c]	8.67 [c]	10.67 [b]	11.33 [ab]	12.00 [a]	0.43	*
ST (mm)[6]:							
22-26 Weeks	0.43 [b]	0.47 [a]	0.46 [a]	0.47 [a]	0.45 [a]	0.01	*
26-30 Weeks	0.40b	0.45a	0.45a	0.46a	0.45a	0.01	NS
30-34 Weeks	0.40 [b]	0.46[a]	0.46 [a]	0.47 [a]	0.47 [a]	0.01	*
HU:[7]							
22-26 Weeks	82.14 [b]	81.72 [b]	84.11 [ab]	85.78 [a]	84.44 [ab]	0.54	*
26-30 Weeks	83.42	82.11	82.83	84.21	84.95	0.45	NS
30-34 Weeks	82.51	84.36	81.76	85.36	82.23	0.63	NS
E.COH (mg/g):[8]							
22-26 Weeks	4.44 [a]	4.08 [b]	4.05 [b]	3.99 [b]	4.06 [b]	0.05	*
26-30 Weeks	4.14 [a]	3.93 [b]	3.91 [b]	3.74 [c]	3.79 [c]	0.04	*
30-34 Weeks	4.13 [a]	3.93 [b]	3.84 [bc]	3.75 [c]	3.73 [c]	0.04	*

[abc] Means with different superscripts within the same row are significantly different ($P \leq 0.05$)

1-AW= albumin weight, 2-YW= Yolk weight, 3- SW=Shell weight, 4-YI =yolk index, 5-YC= Yolk color, 6=ST=Shell thickness, 7=HU= Haugh unit, 8-Egg cholesterol

DISSCUSION

As shown in Table (2,3,4 and 5), the results of the proximate chemical compositions of MOLP revealed that MOLP is an excellent source of protein (amino acid), minerals, vitamins, and different bio-active components such as alkaloids, glycoside, flavonoids, saponins, tannins, triterpenes and coumarins, which are involved in enhancing long-term health benefits (Enwa *et al.,* 2013). The present results indicated that *MOLP* nutritionally adequate and are rich in its content of both essential and non essential amino acid required for improving laying performance. However, MOLP contain a considerable amount of various nutrients, and have been suggested as a good supplement for protein, fiber and minerals (Jongrungruangchok *et al.,* 2010). Clearly, the high nutritional composition of the Moringa plays a significant role in nutritional, medicinal and therapeutic values (Al-Kharusi *et al.,* 2009). Interestingly, the percentage of moisture, CP, CF and ash contents were partially similar with those obtained by Sodamade *et al.* (2013) found that Moringa leaves contains 9.0, 25.37% 17.41 and 5.89 based on dry weight for CP, CF and ash contents. However, the results of Moyo et al. (2011) have detected 19 - 20 natural amino acids, where the highest amino acid percent is that of alanine (3.03%) and the lowest that of cysteine (0.01%). Therefore, it is advised that *Moringa* can be used in diet to improving poultry performances (Abou-Sekken, 2015). We revealed that MOLP has lot of essential minerals that are very useful for bones and egg production among which, calcium is considered as one of the important minerals required for egg production. This is confirmed by Oluwole *et al.* (2013) who reported that Moringa leaves are a very promising source for essential elements. Also, Nzikou *et al.* (2009) reported that Moringa contains calcium, magnesium, potassium and sodium values of 83.75, 251, 36.53 and 22.5 mg /100g respectively. Obviously, Moringa contained reasonable concentrations of vitamins could play an important role in improving health. Clearly, the MOLP might be regarded as a promising candidate as a natural plant rich in bioactive components such as flavonoids and other bio-active components that have been demonstrated to have significant antioxidant activity (Sreelatha and Padma, 2009) which can scavenge free radicals, and combat pathological disorders generated by reactive oxygen species (Mishra *et al.,* 2007).

Productive performance: It is well known that reproductive performance depends on macronutrient contents of the diet and feed intake as well as digestion and absorption processes (Swennen *et al.,* 2007). It is interesting to note that final body weight of hens significantly increased due to the addition of MOLP to their diets. These results are in harmony with the finding of Dey and De (2013) who found that the addition of Moringa at 0.25 or 0.40 % in broiler diets gave a significant improvement in body weight as compared to control. However, egg production was significantly affected by the inclusion of MOLP in the diets during the periods of 26-29 and 30-33 weeks, where the highest values was achieved by hens fed 1.0 % MOLP diet. This may be attributed to the relatively high nutritive value of Moringa (Etches, 1996). Clearly, the addition of MOLP up to 1.5%

significantly increased egg weight, egg mass and improved FCR. The increase of these traits might be due to the balanced nutrient supply and the presence of lysine and methionine and a combination of other amino acids, which might met the required amount of essential nutrients for better production (Bunchasak and Silapasorn, 2005), where increased methionine and lysine in the feed improves egg production and increases egg weight (Fakhraei *et al.,* 2010). The present finding agree with those Ebenebe *et al.* (2012) who reported that chicks fed on Moringa performed significantly better than the birds of control. While, feed intake significantly decreased with increasing addition of MOLP in the diet, this may be attributed bitter taste, therefore, the inclusion of Moringa in the diets could have resulted in reduced palatability and thus reduce feed intake (Onunkwo and George, 2015). It is obliviously the beneficial effect of MOLP on improving FCR might be contributed to different bio-active substrates, which inhibit harmful bacteria, subsequently increased nutrient absorption. This finding are coincided with Abou-Elezz (2012) indicated that Moringa could be used successfully as sustainable tropical feed resource for Rhode Island Red to improved feed conversion ratio. There has been an increased interest in the utilization of the Moringa in improving of poultry performances (AbouSekken, 2015). This may be attributed to that, birds fed Moringa based diets adequately utilized the nutrients they consumed. However, no mortality observed among the experimental groups along the current study. The absence of death cases among the treatments might be due to anti-microbial and availability of vitamins, proteins and minerals in Moringa plant, besides the good house management during the experiment. In addition, the presence of antioxidants in MOLP, reduced mortality might be due to its ability to enhance the immune system of animals (Yang *et al.,* 2006). This is confirmed by Dey and De (2013) noted that dietary Moringa at levels 0.25 or 0.40 % significantly reduced mortality rate compared to control. The current results suggested that supplementation of MOLP up to 2.0% was safe and did not have negative effect on productive traits, where this result indicate that birds fed MOLP might increase egg production, egg weight and improved FCR.

Blood constituents: It is often very difficult to assess the current health status of animals without detailed examination of blood, where examination provides the opportunity to clinically investigate the presence of several metabolities and other constituents in the body and it plays a vital role in the physiological, nutritional and pathological status of the animal (Doyle, 2006). The current results indicated that there were improvements in most biochemical blood parameters measured at the end of laying experiment due to the addition of MOLP, where the more pronounced improvements detected for birds fed 2.0% MOLP. Clearly, the improvement in protein fractions may attributed to MOLP having various phytochemicals and bioactive components such as trace metal ions, vitamins, alkaloids, carotenoids, polyphenols, fats, carbohydrates, and proteins are involved in enhancing long-term health benefits (Sravanthi and Rao, 2014). Moreover, total plasma protein has been reported as an indication of the protein retained in the animal body (Akinola and Abiola, 1991). The relatively greater protein profile of birds receiving dietary MOLP might be an indication of the good protein content and /or quality of the leaf meal. While, A/G ratio appeared to be decreased, and this means that immunity of birds fed different MOLP additives was improved compared to the control group. These results indicate that birds fed MOLP up to 2.0% level might increase protein retention, which would improve laying performance and egg quality. This result coincided with Kout Elkloub (2015) showed that total protein was significantly increased of Japanese quail when fed diet supplemented with 0.2 and 0.4% Moringa as compared to those treated with 0.6% or control group. However, the current data indicated that the addition of MOLP exhibited significant decreases for lipid profile, while plasma HDL significantly increased compared to control one, may attribute to the hypolipidemic effect is due to bioactive phytoconstituent, i.e. alkaloids and saponins (Dong *et al.,* 2007). This agreed with Dey and De (2013) found that the addition of Moringa at 0.25 or 0.40 % in broiler diets reduced total cholesterol, triglyceride and increase HDL. However, either AST or ALT insignificantly affected due to inclusion MOLP. It is well known that liver contain enzymes like ALT and AST, it releases these enzymes to the blood when damaged (Sherwin, 2003), but AST is considered less specific indicator of liver function than other enzymes since it can also be found in many peripheral tissues (in particular the muscles) and hence as a very wide variability (Bovera *et al.,* 2007). Hence, the absence of significant differences among treatment in the present study may reflect normal liver function. These findings are partially agreed with Makanjuola *et al.* (2014) found that the inclusion of Moringa at 0.2%, 0.4% and 0.6 did not influence AST, but ALT significant decrease. However, in our study, the values of T3 and T4 significantly increased as the increase level MOLP in the diet. It is observed that T3 the metabolically active thyroid hormone, plays an active role in energy metabolism and metabolic rate. The increase level of free T3 and T4, reflected the positive effect of MOLP, due to the high content of antioxidant particularly vitamin C and bio-active components, which enhance the physiological responses. This finding agrees with Hassan *et al.* (2015) indicated that T3 and T4 were significantly increased with increase Moringa level from 0.1, 0.2 and 0.3% in broiler diet. While, corticosterone concentration markedly decreased by the increase MOLP in the diets. This may attributed to the action of bio-active components and the antioxidant compounds present in the plant, one of the most important physiological roles that can protect organisms against the deleterious effects of oxidation (Ferreira *et al.,* 2008). Generally, inclusion of MOLP in the diets appeared safe, as they did not adversely affect birds' physiology. Furthermore, the presence of essential nutrients and minerals in Moringa leaves imply that they could be utilized to improve growth performance and health status of poultry (Ogbe and Affiku, 2012).

Egg quality: It is interesting to note that the proportions of components of fresh egg are 32% yolk, 58% albumen and 10 % shell. In the current study it is interesting to note that production of eggs with high albumen and low yolk proportions is implying relatively lower concentrations of cholesterol which is a good quality for egg consumers who prefer eggs obtained from birds fed MOLP. In the current study, inclusion of MOLP had beneficial effect on most egg quality traits especially concerning shell thickness and yolk color. The increased albumin percentage may be attributed to MOLP contains higher amounts of essential nutrients such as amino acids, which can increase albumen percentage. Therefore, good egg shell quality is of major economic concern to commercial egg producers. Clearly, shell thickness increased due to addition of MOLP in the diet. This may be attribute to MOLP inclusion enhanced utilization of Ca or P and its deposition. Shell weight

percentage and thickness which are important for the transportation of the eggs were significantly increased due to the inclusion of MOLP in the diets (El-Sheikh *et al.,* 2015). The Haugh unit is important item in evaluating albumen quality of egg. Moreover, the Haugh unit is considered a standard measure of quality (Williams, 1992). It was realized from the present results that egg quality improved due to addition of MOLP, this may be attributed the higher nutritive value of MOLP (Sarwatt *et al.,* 2004). On the other hand, different bio-active components present in MOLP that enhance digestion and absorption of different nutrients may be responsible for improved egg traits. Further, yolk color is a preferable trait for consumer, where yolk color improved due to the addition of MOLP, this may be attributed to the fact that MOLP is rich in beta-carotene, oxycaretenoids and xanthophyll content, which are a good source for yolk pigments (Abou-Elezz *et al.,* 2012). This finding agree with that of Lu *et al.* (2016) who found that supplementary Moringa at 5% and 10% improved the albumen height and yolk color of storage eggs. In general, Ebenebe *et al.* (2012) indicated that inclusion of Moringa at different levels, (0%, 2.5%, 5.0% and 7.5%), improved egg quality at low level but high levels resulted in poorer egg quality indices of Isa Brown chicken. Concerning the cholesterol in eggs, it is well known that there is no cholesterol in the white part of an egg, but it is found in the yolk. As a matter of fact, in laying hens, egg cholesterol is synthesized in the liver and secreted into the blood as very low density lipoprotein (VLDL) particles, the main yolk cholesterol carrying macromolecules. Plasma VLDL particles are then internalized by the oocyte vitellogen in receptor in the rapidly growing follicles and deposited to yolk. Thus, cholesterol is mainly excreted through the egg in the hen. Faecal neutral and acidic sterol represents a second major pathway for elimination of cholesterol (Hall and Mckay, 1993). It is revealed that the addition of MOLP in diets of laying hens provoked a significant reduction in cholesterol contents of eggs, may be due to bio-active components such as alkaloids, saponins and etc., that have cholesterol lowering activities. These phytochemicals are the major secondary metabolites present in the MOLP holds some therapeutic potential for chronic hyperlipidemia (Fahey, 2005). Obviously, MOLP contains high fiber, which binds cholesterol and inhibits its intestinal absorption until it is eliminated, thus reducing either cholesterol or LDL, where the main function of insoluble fiber is to bind bile acids, which reduces fat and cholesterol absorption (Joshi and Mehta, 2010). The present finding are consistent with those of *Lala et al. (2012) who showed* that egg cholesterol and LDL reduced with MOLM supplementation in layers diet.

In conclusion, based on the findings of the present study MOLP is well tolerated by the Lohmann layer chickens, and it has potential benefits as a feed additive in improving productive and physiological status. Therefore egg producer can incorporating Moringa up to 2% as a safe feed additive to laying hens to attain high productive and physiological performance.

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تعظيم إنتاجية سلالة دجاج اللوهمان بتغذيتها على علائق تحتوي علي مستويات مختلفة من مسحوق أوراق المورينجا كأضافات غذائيه آمنة

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تهدف هذه الدراسة إلي اختبار تأثير اضافه مستويات متدرجة من مسحوق أوراق نبات المورينجا أوليفيرا كإضافات غذائيه آمنة على الأداء الإنتاجي ، بعض مقاييس الدم، خصائص جودة البيض ومحتوى البيض من الكولسترول. إستخدم عدد ١٥٠ دجاجة بياض لوهمان عمر ٢٠ أسبوع حيث تم توزيعها عشوائيا إلى ٥ معاملات غذائية، كل معامله احتوت على ثلاث مكررات. كل مكرره احتوت علي ١٠ دجاجات و كان متوسط الوزن الأولي للجسم من ١٥٠١ إلى ١٥٣٠ جرام حيث تم تسكين كل طائرين في قفص واحد. تم تغذية المجموعة الضابطة على عليقه الكنترول التى احتوت على الذرة وكسب فول الصويا، وغذيت المجموعات التجريبية على عليقه الكنترول مضاف اليها ٠.٥٪ ، ١.٠٪ ، ١.٥ ، ٢.٠٪ من مسحوق أوراق نبات المورينجا أوليفيرا. أظهرت النتائج ان الاداء تحسن معنويا بسبب إضافة مسحوق أوراق نبات المورينجا أوليفيرا مقارنة مع المجموعة الضابطة. علاوة على ذلك سجل معدل التحويل الغذائي قيم أفضل للمجموعات المغذاه علي مسحوق أوراق نبات المورينجا أوليفيرا. تحسن سمك قشرة البيضة وكذلك لون صفار البيض باضافه مسحوق نبات المورينجا. بالإضافة إلى ذلك إنخفض محتوي الكوليسترول في البيض بزيادة مسحوق أوراق نبات المورينجا أوليفيرا في العلائق. كما تحسنت الحالة الصحية التي تم قياسها من خلال قياس بعض مكونات الدم حيث انخفضت نسبة الدهون ومعدل تركيز هرمون الكورتيكوستيرون، في حين أن مستوي الدهن عالي الكثافة و البروتين الكلي قد زاد مع زيادة مسحوق أوراق نبات المورينجا أوليفيرا في العلائق. ويمكن التوصية بأن إضافة مسحوق أوراق نبات المورينجا أوليفيرا في العلائق لدجاج اللوهمان تعزز من اداءها الإنتاجي وتحسن حالتها الفسيولوجيه. ولذلك فان اضافه مسحوق أوراق نبات المورينجا ليس له آثار سلبية علي الأداء الإنتاجي أو الحالة الصحية للدجاج.