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Effect of Dietary Supplementation with *Moringa oleifera* Leaves and/or Seeds Powder on Production, Egg Characteristics, Hatchability and Blood Chemistry of Laying Japanese Quails

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Abstract: The present study aimed to evaluate the effect of dietary *Moringa oleifera (M. oleifera)* leaves and/or seed powder on laying Japanese quail performance in terms of egg production, egg quality, blood serum characteristics, and reproduction. In total, 168 Japanese quails (120 hens and 48 males) at eight weeks of age in laying period were randomly distributed to four treatment groups, with six replicates per group and seven birds (five hens and two males) per replicate. The first group (G1) served as a control group, while G2, G3 and G4 groups were supplemented with *M. oleifera* leaves (ML) and *M. oleifera* seeds (MS) and their combination ((1 g/kg ML; 1 g/kg MS; and 1 ML g/kg + 1 MS g/kg (MSL), respectively). From the results, feed consumption, feed conversion ratio, egg weight, fertility and hatchability from fertile eggs, egg and yolk index, and Haugh unit were not affected by dietary treatments. However, egg production, egg mass, eggshell thickness, and hatchability were significantly increased and blood aspartate transaminase (AST) and urea decreased in the MS treatment. Both triglycerides and total cholesterol were reduced ($p < 0.05$) in all treatments with ML, MS, and MSL, with no significant differences in alanine aminotransferase (ALT), albumin, total protein, globulin, and A/G ratio among dietary treatment. Our results clearly indicated that the inclusion of *M. oleifera* seeds in Japanese quail diet significantly increased egg production and improved hatchability, along with some egg quality parameters, and also lowered some blood biochemical components.

Keywords: *Moringa oleifera*; Japanese quail; fertility; egg production; livestock

1. Introduction

Traditional synthetic feed additives such as antibiotics, growth stimulants, antioxidants, antiparasite, and antifungal agents have been used for decades in poultry feed. However, they pose many issues such as residues in animal products and resistance to antibiotics in the consumer, which is a matter of

public health [1]. Therefore, the use of antibiotics as a growth stimulant in animal feed was banned in Europe. Revolution in animal feed production has resulted in the development of feed additives in the forms of phytogenics [2,3]. Herbs and their metabolites (known as bioactive substances) play a good role as feed additives. These bioactive compounds such as carotenoids, flavonoids, and herbal oils help enhance animal health and productivity to yield safe and healthy products [4]. The essential role of these active compounds is to dampen microbes and toxins in the gut and promote effectiveness of the pancreas, resulting in good metabolism of nutrients [3,5]. Medicinal plants contain several phytochemicals and bioactive compounds such as trace metal ions, alkaloids, vitamins, carotenoids, fats, polyphenols, carbohydrates, and proteins, which are useful for long-term health [6]. Compared to antibiotics, the utilization of plants elevates trust of usage. Herbs have been suggested to enhance metabolic processes and the health conditions of livestock [7]. Several plants may improve the effect of digestive enzymes, feed consumption, feed utilization, and carcass traits [8]. However, Halle et al. [9] did not observe significant effects for some additives such as oregano and its essential oils, savory, *Nigella sativa* L. and cacao husks on live weight, and carcass parameters of broilers.

M. oleifera plays a useful role against inflammatory and oxidant effects [10]. The administration of *M. oleifera* leaf extracts hinders the development of pathogenic gram-positive and gram-negative bacteria and antioxidant activity. There was an improvement in Hubbard broiler chicks' performances, immune response, and carcass quality parameters with increased benefits with the usage of *M. oleifera* [11]. The growing popularity of using *M. oleifera* as a feed additive in poultry nutrition necessitates thorough investigation into its nutritional value, as well as its effects on hematological characteristics as a measure of both the nutritional and medicinal importance of its leaves in broiler chicks. It was indicated that many vitamins (A, E, B2, B5, B6, folic acid) and minerals (Ca, Fe) are present in moringa [12], having also a powerful fungicidal and antimicrobial activity. It also has an inhibitory effect on cholesterol levels in blood [13]. Yang et al. [10] indicated that *M. oleifera* improved immunity, lowered *E. coli*, and enhanced Lactobacilli in the gastrointestinal tract of chickens. *M. oleifera* improves feed conversion ratio and increases the immune reception of birds [13]. It also has natural antioxidant components and dissolvable proteins in its leaves [14]. Elkloub et al. [15] found that abdominal fat and plasma cholesterol, especially low-density lipoprotein (LDL), decreased with improved performance of immune organs and blood constituents using *M. oleifera* leaves meal in Japanese quail diets. There are only a few studies on the bioactive constituents of *M. oleifera* leaves and their effect on meat antioxidant status [15,16]. Therefore, the objective of this study was to verify the usefulness of *M. oleifera* leaves and/or *M. oleifera* seed meal as natural feed supplements and as a source of antioxidants on the productive and physiological characteristics of laying Japanese quails.

2. Materials and Methods

The experiment was performed at the Research Farm, Poultry Department, Faculty of Agriculture, Zagazig University in Egypt. All research protocols were conducted with the approval of the Local Experimental Animal Committee and were confirmed by the organized council.

2.1. Analysis of M. oleifera

Moringa oleifera plants have high amounts of crude protein (CP) in the leaves (251 g/kg) dry matter (DM) and an abundant proportion of tannins and some anti-nutrient components and offers an abundant source of proteins for ruminants and non-ruminants [17]. The nutrient structure of *M. oleifera* seeds and leaves were determined according to the Association of Official Analytical Chemists (AOAC) [18], as shown in Table 1. The content of total phenolic compounds and total flavonoids in *M. oleifera* seed and leaf mixture were estimated according to Gurnani et al. [19] and Meda et al. [20], respectively as shown in Table 1.

Table 1. Determined analysis *of M. oleifera* seeds and leaves.

Items (g/kg)	Dry Matter	Ash	Crude Protein	Ether Extract	Crude Fiber
Seeds	965	33.9	395.8	394	46
Leaves	934	137.5	270.1	62	215
Content of total phenolics and total flavonoids in seed and leaf mixture					
Total phenolics (mg GAE/g)			65.24		
Total flavonoids (mg QE/g)			17.58		

GAE: gallic acid equivalent; QE: quercetin equivalent.

2.2. Birds, Experimental Design, and Diet

In total, 168 Japanese quails (120 hens and 48 males) at eight weeks of age in laying phase were randomly divided into four treatment groups in a complete randomized design experiment with four treatments having six replicates of seven birds (five hens and two males) each. The experiment included three dietary treatments including *M. oleifera* leaves (ML), *M. oleifera* seeds (MS) and their combination with a level 1.0 g/kg diet of ML; MS; and ML + MS (MSL), and a basal control-diet without moringa. *Moringa oleifera* leaves (ML) and *M. oleifera* seeds (MS) were purchased from the local market, Egypt. The experimental period spanned from 8 to 20 weeks of age. The birds were housed in 24 cages, and each cage measured 90 × 40 × 40 cm. Each metallic cage had a drinker in the form of nipples and feeders. The light program was 14 h of light daily at the start of the experiments and was increased by 15 min weekly to 16 h of light. The birds were allowed to eat and drink ad libitum at every period of the experiment. The corn-soybean diets were in a mash form and were calculated according to the National Research Council (NRC) [21] (Table 2). Birds were vaccinated with distilled water by a veterinarian at the appropriate age. The highest and lowest ambient temperatures were noted every day at noon (12.00 PM) and ranged from 14 to 23 °C, whereas the relative humidity was approximately 60%–70%. All quails were reared in wire batteries under the same managerial, hygienic, and environmental conditions.

Table 2. Composition and calculated analysis of layer quail diet.

Items	Laying Period 8–20 Weeks
Ingredients %	
Yellow corn	55.00
Soybean meal 44% CP	29.50
Corn gluten 60% CP	3.65
Cotton seed oil	4.30
Dicalcium phosphate	1.70
Limestone	5.00
Salt	0.30
Premix *	0.30
L-lysine	0.08
DL-methionine	0.17
Calculated analysis **	
Crude protein %	19.96
Metabolizable energy MJ/kg	12.61
Calcium %	2.51
Available Phosphorous %	0.37
Lysine %	1.02
Methionine %	0.45
Methionine + Cysteine %	0.77

* Layer vitamin and mineral premix. Each 2.5 kg consisted of vit. A. 12 Miu, E. 15 IU., vit. D_3 4 Miu; vit. B_1 1 g, vit. B_2 8 g, pantothenic acid 10.87 g, nicotinic acid 30 g, vit. B_6 2 g, vit. B_{12} 10 mg, folic acid 1 g, biotin 150 mg, copper 5g, iron 5g, manganese 70 g iodine 0.5 g, selenium 0.15 g, zinc 60 g, antioxidant 10 g. ** Calculated according to National Research Council (NRC) [21].

2.3. Collection of Data

Feed utilization was recorded and measured by grams of feed consumed over 28 days and divided by the number of birds/day, and mortality rates were checked. The feed conversion ratio (g feed/g egg) was determined according to the egg mass value divided by the quantity of feed consumption. Eggs were collected every day and egg production was calculated on a hen-day basis. Egg number and egg weight were recorded daily, and the egg mass (egg number × egg weight) was calculated.

2.4. Egg Characteristics

The exterior and interior egg quality parameters were examined. Three eggs from every replicate were collected, and egg components were measured during different time intervals. The shape index of eggs was calculated according the proportion of egg width to length [22]. The yolk index was calculated as yolk height/yolk diameter (mm) after separating the yolk and albumen according to Keener et al. [23]. The shell thickness of eggs was examined (with shell membrane) using a micrometer. The thickness of the shell was measured at three different places on the eggs (air cell, equator, and narrow end). The Haugh unit was calculated as

$$\text{Haugh unit score} = 100 \times \log (H + 7.57 - 1.7\ W\ 0.37)$$

where H is the height of albumen and W is the weight of egg, as per the formula proposed by Card and Nesheim [24].

2.5. Fertility and Hatchability Percentages

In total, 45 eggs from each treatment were collected after three weeks of the experimental period. Eggs were then sprayed with TH4® solution (2 ml/liter of water) for disinfection and set in an incubator. The incubated eggs were subjected to 37.5 °C and 65% RH for the first 14 days. The eggs were transferred to a hatchery machine at the end of the 14th day of incubation and received 37.4 °C and 70% RH until hatching. After hatching, the chicks were counted and the eggs that were not hatched were counted to calculate the fertility rate (number of hatched chicks + number of fertile non hatched eggs/total number of eggs set in incubator) × 100 and hatchability percent. The hatchability was expressed according to the chicks that hatched from fertile eggs.

At the end of the trial, blood samples were collected from six hens, which had been randomly chosen from each group for slaughter, with a clean antiseptic pipe. Samples were allowed to clot and were centrifuged at 3500 rpm (G-force value = 2328.24) for 15 min to obtain serum. Serum samples were preserved in Eppendorf tubes at −20 °C until further examination. Blood biochemical characteristics were specified as total protein (TP), albumin (ALB), globulin (GLB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, creatinine, urea levels, total cholesterol (TC) and high density lipoprotein (HDL), cholesterol, triglyceride (TG) and measured spectrophotometrically using commercial kits provided by Biodiagnostic Co. (Giza, Egypt). Low-density lipoprotein (LDL) cholesterol was calculated as described by Friedewald et al. [25].

2.6. Statistical Analysis

All the statistical analyses of the obtained results were achieved using the SPSS software program [26]. The average values and standard error of the mean (SEM) are described. All data were evaluated with a one-way analysis of variance (with the diet as the fixed factor) using the post-hoc Newman-Keuls test, and $p < 0.05$ was considered to be statistically significant.

3. Results and Discussion

3.1. Effect of M. oleifera on Productive Performance

Effects of *M. oleifera* on production performance are presented in Table 3. Feed consumption, feed conversion ratio, and egg weight were not affected by dietary *moringa* leaves, seeds, and their combination. However, egg production and egg mass were increased significantly using MS compared to the other groups. Kwariet et al. [27] found no significant effects for *M. oleifera* leaf meal at a level of 1%–2% of the basal diet on feed conversion and egg weight of Vanaraja laying hens. This is not in agreement with Olugbemi et al. [28] who found that using Moringa leaf meal (20%) as a replacement for sunflower seed meal in chicken layer diets led to significant decrease in egg production and whole egg weight. Riry et al. [29] found that feeding Japanese quails on a diet with 5% *M. oleifera* seed meal led to a decrease in feed intake in contrast to the control birds. Authors of previous studies [28,30] postulated that the use of ML up to 10% had no negative effects on the egg production of laying birds, but levels greater than 10% led to adverse effects possibly due to increasing the level of anti-nutritional factors and dustiness of ML and low digestibility of energy and protein.

Table 3. Production performance of laying Japanese quails as affected by dietary treatments.

Items	Control	MS	ML	MSL	SEM	*p* Value
Feed intake (g/d/bird)	33.54	33.23	33.09	33.67	0.10	0.14
Feed conversion ratio (g/g)	3.18	2.83	3.20	3.02	0.20	0.07
Egg production (%)	78.95 ^{b,c}	83.41 ^{a}	76.93 ^{c}	81.73 ^{a,b}	0.88	0.01
Egg weight (g)	13.39	14.07	13.50	13.63	0.16	0.49
Egg mass (g/d/bird)	10.57 ^{b}	11.74 ^{a}	10.38 ^{b}	11.14 ^{a,b}	0.06	0.03

Control, the basal diet; MS, 1 g *Moringa* seeds/kg diet; ML, 1 g *Moringa* leaves/kg diet; MSL, 1 g seeds + 1 g leaves/kg. SEM: standard error means. a–d: Means in the same row with no superscript letters after them or with a common superscript letter following them are not significantly different ($p < 0.05$).

3.2. Effect of M. oleifera on Fertility and Hatchability

Data of fertility and hatchability of the eggs are presented in Table 4. The results showed that fertility and hatchability from fertile eggs were not affected by dietary ML, MS, and their combination. But hatchability was significantly increased by using only MS compared with the other groups. Etalem et al. [31] found that the hatchability percentage in the 5% ML group was significantly higher than that of the control. Mahmood and Al-Daraji [32] and Moyo et al. [33] observed that *M. oleifera* leaves have higher levels of zinc and vitamin E, which can be useful to the hatchability of eggs.

Table 4. Fertility and hatchability of eggs from laying Japanese quails as affected by dietary treatments.

Items	Control	MS	ML	MSL	SEM	*p* Value
Fertility (%)	90.35	92.78	89.18	89.95	0.77	0.42
Hatchability (%)	64.62 ^{b}	71.87 ^{a}	66.24 ^{b}	68.03 ^{a,b}	1.02	0.04
Hatchability (fertile eggs, %)	71.59	77.54	74.29	75.66	1.10	0.30

Control, the basal diet; MS, 1 g Moringa seeds/kg diet; ML, 1 g Moringa leaves/kg diet; MSL, 1 g seeds + 1 g leaves/kg. SEM: standard error means. a, b: Means in the same row with no superscript letters after them or with a common superscript letter following them are not significantly different ($p < 0.05$).

3.3. Effect of M. oleifera on Egg Quality

The results in Table 5 show the effect of *M. oleifera* on egg characteristics. Results showed that shell thickness was significantly increased by adding MS meal compared to the control diet. Whereas, egg index, shell percent, yolk percent, albumen percent, Haugh unit, and yolk index were not affected by the dietary supplementation. There was no difference in Haugh units, eggshell strength, or egg shape index among the groups ($p > 0.05$) in response to dietary *M. oleifera* leaves [34–36]. Ebenebe et al [37]

reported that adding MOL had no effect on egg shape index that's correlated with the strength of an eggshell and the grade of eggs. Mabusels et al. [38] found that the addition of *M. oleifera* seed meal to layer diets increased the shell thickness ($p \leq 0.05$), compared to a diet with 10% *Moringa oleifera* seed meal (MOSM) and the control diet. However, both 5% and 7.5% MOSM supplementation was equally effective for eggshell thickness. Generally, the seed contains antioxidants, essential oils, minerals such as Ca, Mg, K, Se, P, and Zn, and vitamins such as A, C, D, K, and E, so it can improve egg quality and improve most of the egg quality parameters. The egg shape index percentage was significantly reduced ($p < 0.05$) in birds fed diets containing 10% MS [39].

Table 5. Egg quality criteria for laying Japanese quails as affected by dietary treatments.

Items	Control	MS	ML	MSL	SEM	*p* Value
Egg index	75.74	79.46	79.46	80.27	0.84	0.24
Shell, %	13.38	13.22	13.45	13.61	0.12	0.75
Yolk, %	32.40	31.64	32.62	31.78	0.24	0.44
Albumen, %	54.23	55.14	53.93	54.61	0.24	0.32
Shell thickness, mm	0.23 ^{b}	0.26 ^{a}	0.25 ^{a,b}	0.25 ^{a,b}	0.004	0.03
Haugh unit	92.57	95.05	93.87	94.39	0.81	0.79
Yolk index	48.66	49.33	49.06	48.83	0.46	0.97

Control, the basal diet; MS, 1 g Moringa seeds/kg diet; ML, 1 g Moringa leaves/kg diet; MSL, 1 g seeds + 1 g leaves/kg. SEM: standard error means. a, b: Means in the same row with no superscript letters after them or with a common superscript letter following them are not significantly different ($p < 0.05$).

3.4. Effect of M. oleifera on Blood Biochemical Indices

Data of blood biochemical indices for laying Japanese quails are listed in Table 6. The results showed that AST (U/L) was significantly decreased due to ML and MS in comparison to the control group and MSL. The urea level was lowered by only *Moringa* seeds (MS) having a powerful fungicidal and antimicrobial activity. Further, both triglycerides and cholesterol were significantly reduced by all dietary treatments including ML, MS, and MSL compared with the control group. ALT, albumin, total protein, globulin, and the albumin/ globulin ratio (A/G) were not affected due to any dietary treatment. Laying hens in the ML group had a lower concentration of albumen (ALB) and urea (UA) than those in the control group ($p < 0.05$) [36]. AST decreased with *M. oleifera* supplementation. The birds fed *M. oleifera* (ML and MS) recorded significantly ($p \leq 0.05$) reduced cholesterol levels. The reduction in cholesterol levels may be because *M. oleifera* contains hypocholesterolemic agents such the phytoconstituents and β-sitosterol [39]. Yuangsoi et al. [40] found that the levels of ALT and AST were similar in all diets, indicating normal organ function upon feeding with *Moringa* seed meal. Elkloub et al. [15] reported that plasma AST and ALT decreased with all levels of ML. As the liver produces enzymes like ALT and AST and releases them into the blood upon liver damage [37], thus, the absence of significant differences in serum AST values may indicate normal liver function of the birds on diets containing MSL. Lowered AST activity was observed in hens on 0.4% and 0.6% ML, which could suggest that ML can elevate liver health as well. Makanjuola et al. [38] observed that 0.2%, 0.4%, and 0.6% ML did not affect serum total protein, albumin, globulin, and AST levels. However, AST showed significant reduction in the birds fed a diet of (0.4%) *M. oleifera* leaves, with a good effect on the immune responses and development of the intestinal health of birds [28].

Table 6. Blood biochemical indices of laying Japanese quails as affected by dietary treatments.

Items	Control	MS	ML	MSL	SEM	*p* Value
AST (U/L)	193.00 [a]	134.00 [b]	144.00 [b]	178.67 [a]	8.50	0.01
ALT (U/L)	57.00	54.67	50.67	48.00	2.38	0.61
Total protein (g/dL)	5.80	6.30	6.07	6.23	0.08	0.13
Albumin (g/dL)	3.47	3.33	3.67	3.30	0.07	0.22
Globulin (g/dL)	2.33	2.97	2.40	2.93	0.12	0.09
Albumin / Globulin ratio	1.49	1.13	1.56	1.16	0.08	0.12
Triglycerides (mg/dL)	65.33 [a]	27.00 [b]	36.00 [b]	35.33 [b]	4.59	0.0002
Cholesterol (mg/dL)	115.33 [a]	47.67 [c]	85.00 [b]	74.67 [b]	7.76	0.0004
Urea (mg/dL)	59.67 [a]	41.67 [b]	58.33 [a]	46.00 [a,b]	2.92	0.03
Creatinine (mg/dL)	0.70	0.53	0.70	0.60	0.04	0.35
Bilirubin (mmol/L)	0.37	0.60	0.43	0.37	0.04	0.14

Control, the basal diet; MS, 1 g Moringa seeds/kg diet; ML, 1 g Moringa leaves/kg diet; MSL, 1 g seeds + 1 g leaves/kg. SEM: standard error means. a,b: Means in the same row with no superscript letters after them or with a common superscript letter following them are not significantly different ($p < 0.05$).

4. Conclusions

In comparison with the control group, egg production, egg mass, hatchability (%), and shell thickness were significantly higher and blood urea, AST, and lipid profile (cholesterol, and triglycerides) concentrations were significantly lower in birds fed a *M. oleifera* seed supplemented diet (treatment MS). From our results, we recommend the use of Moringa seeds at 1 g/kg in the diet of laying Japanese quails.

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