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Research Article

Efficacy of Oil Mixture Supplementation on Productive and Physiological Changes of Laying Japanese Quail (*Coturnix coturnix japonica*)

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Abstract

The objective of this study was to determine the optimal level of an Oil Mixture (OM) supplementation in drinking water to enhance the performance of older Japanese quail. Five hundred forty Japanese quail, 40 weeks old, were randomly assigned to 4 experimental groups (90 female and 45 male/group) that received OM at 0, 0.5, 1 and 2 mL L^{-1} of drinking water during the experimental period of 42 days. Egg production, egg quality, fertility and hatchability percentages were evaluated. Serum alanine aminotransferase (ALT), aspartate transferase (AST), total protein, albumin, urea, creatinine, total lipids, cholesterol and triglycerides were estimated. Livers were examined for histopathological changes. Results showed that birds received 0.5 mL L^{-1} of OM had significantly improved in most studied traits compared to the other treated and control groups. Laying rate, fertility, hatchability percentage, yolk index, internal quality unit and ovarian yellow follicle number were superior in 0.5 group than the other experimental groups. In contrast, birds received 1 or 2 mL had significantly higher concentrations of ALT and AST than birds that received 0.5 mL or control birds. Serum chemistry analysis revealed no significant effects due to treatments on kidney functions. Histopathological findings revealed disruption of normal hepatic architecture in birds that received 1 or 2 mL of OM supplementation compared to 0.5 mL and control birds. Our findings suggest that 0.5 mL L^{-1} of OM could be enough and useful in improving productive and physiological performance of laying Japanese quail.

Key words: *Moringa oleifera*, heat stress, broiler performance, blood parameters, carcass characteristics

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Medicinal plants have gained interest in alternative feed strategies for the future. These plants and their products including plant extracts have been adopted in poultry rations to enhance growth performance and to improve feed intake and efficiency (Abd El-Latif *et al*., 2013). Oil Mixtures (OM) are complex mixtures of secondary metabolites of these plants containing bioactive compounds and consisting of low boiling point phenylpropenes and terpenes (Greathead, 2003). They have shown some beneficial activities such as antioxidant (Botsoglou *et al*., 2002), enzymatic effects (Hernandez *et al*., 2004), digestion improving (Alcicek *et al*., 2004), anti-heat stress and immune system responsiveness (Cabuk *et al*., 2006). Other workers investigated the effects of OM on egg shell quality of laying hens (Bozkurt *et al*., 2009) and productive and reproductive performance in broiler breeders (Bozkurt *et al*., 2009).

Recently, scientists postulated that in the near future, OM will play a huge role in the poultry industry (Gopi *et al*., 2014). Because many OM compounds have strong antimicrobial activity, research to exploit OM as feed additives in poultry nutrition has been accelerated due to the ban of some antibiotic as growth promoters in many developed countries. Generally, they are very potent molecules and must be used in small quantities. Adversely they can affect the function of digestive system can cause allergies, suppress feed intake and can be stored in tissues (Peric *et al*., 2010). However, there is little published data on the optimum levels and period of usage of OM. Therefore, further research is needed to explore in detail and establish the optimal application of such additives including their optimal dosage level in order to obtain maximum effects. The main objective of this study was to investigate the efficacy of OM containing a mixture of nine oils as a supplement in drinking water on productive and physiological performance of laying Japanese quail.

MATERIALS AND METHODS

Birds housing and treatments: The present study was carried out in the poultry farm of the Department of Animal Production, Faculty of Agriculture, Suez Canal University Ismailia, Egypt. Five hundred forty weeks old Japanese quails were randomly assigned to 4 experimental groups (90 female and 45 male/group) first group served as the control non-supplemented group, while the other groups received 0.5, 1, 2 mL L^{-1} of an OM in their drinking water for a period of 6 weeks. Birds were fed a conventional corn and soybean meal basal diet, formulated to meet all the nutritional requirements of laying quail according to specifications of the NRC (1994). They were housed in floor pens (2×2 m) and provided with 16 h light/day throughout the experiment period of 42 days. Feed and water were provided *ad libitum*. Oil mixture commercially registered under the name of "Golden rose" was generously provided by A.M. Treding Co., Alexandria, Egypt. The product is a mixture of the followings: sweet almond oil (20%), olive oil (10%), soya bean oil (5%), lavender oil (15%), eucalyptus oil (5%), coconut oil (15%), pepper mint oil (10%), sesame seed oil (7%) and citrus oil (13%). The chemical composition of the oil mixture was analyzed by using gas chromatography coupled with mass spectrometry (GC/MS) which revealed that OM could be classified into two main groups of chemical constituents including the hydrocarbons (which composed exclusively of terpenes mainly monoterpenes) and the oxygenated compounds (which including esters, aldehydes, ketones, alcohols, phenols and oxides). Therefore, seventy compounds were identified which comprised 100% of the total constituents and the main compounds (represented more than 70% of total constituents) were identified as following: Anethole (16.69%), Limonene (14.78%), Eucalyptol cineole (10.55%), Glycerol trilaurate-glycerol tridodecanoate (10.21%), Diporpylene glycol 1- propanol, 2-2 hydroxypropyl (2.73%), isomenthone (2.13%), Trans-Traumatic acid (2.11%), 2-propanol -1,1 oxybis-dipropyline glycol (2.04%), Menthol (1.76%), Dicyclohexo-8-crown 6-dicyclohexyl -18-crown 6 (1.70%), Eugenol (1.38%), Alpha pinene (1.24%), Beta pinene (1.24%), Diethyl phthalate (0.85%).

Data collection

Productive and reproductive traits: Body weights of all birds were recorded at zero time, 3 and 6 weeks of experimental period. Daily egg number and weekly egg weights were recorded. Laying rate and egg mass were calculated throughout the experimental period. A total of 1750 hatching eggs from all experimental groups were used to determine fertility and hatchability percentages at 3 and 6 weeks from experiment (3 replicate each). Eggs were collected daily and stored at 18°C and 65 RH% up to 5 days before they were placed in the hatchery. At hatching, all live and dead chicks were counted. The un-hatched eggs were opened and classified either as being infertile or embryonic death. The embryonic mortality was classified according to Khalil *et al*. (2016). A total of 480 quail eggs from all experimental groups were used to measure egg quality traits for two consecutive days at 3 and 6 weeks of experimental period. Egg shape index, internal quality unit, yolk shape index and shell thickness were calculated. Mortality rates among birds were recorded during the experimental period.

Reproductive organs and biochemical parameters: At three weeks of the experimental period blood samples were collected for biochemical parameters analysis, samples were obtained from 10 birds (5 males and 5 females) from each group by heart puncture. At the end of experiment, 40 birds from all experimental groups (20 males and 20 females) were sacrificed. Blood samples at 3 and 6 weeks of experiment were collected in plain tubes without anticoagulant, centrifuged at 3000 rpm for 15 min and serum obtained was stored at -20°C for further analysis. Serum ALT, AST, total protein, albumin, urea, creatinine, total lipids, cholesterol and triglycerides were determined with commercial kits from Spinreact Company (Spain) as per manufacturer instructions. Ovarian morphology was studied and numbers of yellow follicles were counted. Absolute and relative weights of ovaries, oviducts and testes were determined.

Histopathology of the liver: Liver specimens from 5 sacrificed birds from each group were immediately removed, fixed in 10% neutral formalin saline, dehydrated in serially ascending alcohol solutions and then embedded in paraffin blocks. They were sectioned and stained with hematoxylin and eosin and were examined microscopically for histopathological changes.

Statistical analysis: Data were analyzed using the General Linear Model (GLM) procedure of SAS (SAS., 2001). Differences among means were detected using Duncan's new multiple test (Duncan, 1955). Correlation coefficients among traits were estimated.

RESULTS

Egg production traits: The percentages of hen-day egg production were different ($p \leq 0.05$) among treatment groups at 3, 4, 5 and 6 weeks from treatments as shown in Fig. 1. Groups were supplemented with OM showed rapid increase in egg production rate up to 3 weeks compared with control group. Thereafter, treated groups behaved differently according to level of OM and varied significantly during the remaining period of experiment. Birds supplemented with 0.5 mL had almost relatively constant high production ($p \leq 0.05$). On the other hand, birds supplemented with 2 mL showed a trend toward decreased production at week 4 and significantly decreased ($p \leq 0.05$) at weeks 5 and 6 compared with control group. Also birds supplemented with 1 mL showed decreased production ($p \leq 0.05$) at weeks 4 and 5, while its production rate increased ($p \leq 0.05$) at week 6 as compared to controls. In the same context, Laying Rate (LR), Egg Number (EN), Egg Weight (EW) and Egg Mass (EM) were affected.

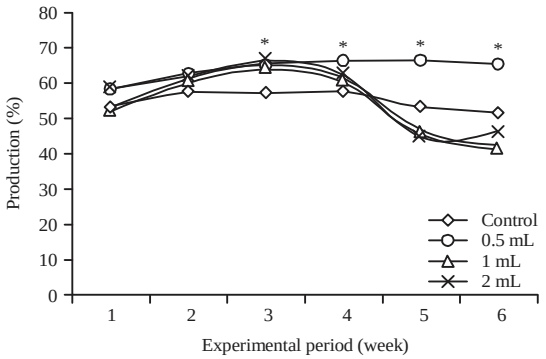

Fig. 1: Hen-day egg production of laying Japanese quail supplemented with different levels of OM

During the 1st period (up to three weeks) results showed a marked improvement in LR and EN in OM treated groups, which was significant in 0.5 and 2 mL when compared to the control and showed a trend toward increase when compared to 1 mL treated group. Meanwhile, supplemented with 0.5 mL OM caused significantly higher LR and EN when compared with other treated and control, during the 2nd (from third till sixth weeks) and the entire periods (Table 1).

Birds supplemented with 0.5 mL were significantly lower egg weights than those recorded in other groups during entire periods. In addition, results showed a significant ($p \leq 0.05$) difference among treatments on EM. The highest EM was recorded in 2 mL group. But the lowest value was recorded in 0.5 mL group during the 1st period. In contrast, 0.5 mL group had significantly higher during the 2nd period than other experimental groups and non-significantly higher during entire experimental period except with 1 mL group. Furthermore, LY and EM during the 2nd period were significantly deteriorated in birds received 1 and 2 mL OM compared to the 1st period. In contrast, EM during the 2nd period were significantly improved in birds received 0.5 mL OM compared to the 1st period. On the other hand, No significant effects among treatments on total mortality rates of male and female quails were found throughout the experimental period.

Egg quality: Results in Table 2 showed a significant effect on Egg Shape Index (ESI). Birds supplemented with 0.5 and 1 mL showed significantly higher ESI than control group and non-significantly different as compared to 2 mL group during entire period. Also, birds supplemented with 0.5 mL had higher YI than the other groups during the 1st, 2nd and entire experimental periods.

Data showed significant ($p \leq 0.05$) differences in Internal Quality Unit (IQU) among treatments during experimental periods (Table 2). Birds supplemented with 2 mL recorded the

Table 1: Effects of OM supplementation on egg production traits

	Treatments				
Items	Control	0.5 mL	1 mL	2 mL	Overall
Laying rate (%)					
Period I[1]	56.00±1.54^{b}	62.39±2.71^{a}	59.57±1.71^{aA}	62.69±1.61^{aA}	60.21±1.54^{A}
Period II[2]	54.61±1.49^{b}	66.34±1.31^{a}	49.57±2.66^{bB}	51.00±2.64^{bB}	55.38±2.14^{B}
Overall	55.26±0.93^{b}	64.37±1.53^{a}	54.64±1.74^{b}	56.85±1.77^{b}	
Egg number/hen					
Period I	11.76±0.21^{b}	13.11±0.56^{a}	12.51±0.35^{abA}	13.16±0.33^{aA}	12.66±0.24^{A}
Period II	11.46±0.31^{b}	13.91±0.27^{a}	10.44±0.55^{bB}	10.71±0.55^{bB}	11.61±0.12^{B}
Total	23.22±0.26^{b}	27.02±0.42^{a}	22.95±0.49^{b}	23.87±0.61^{b}	
Egg weight (g)					
Period I	13.75±0.31^{a}	11.58±0.23^{b}	13.16±0.34^{a}	13.38±0.59^{a}	12.95±0.18
Period II	13.52±0.32^{a}	12.12±0.22^{c}	12.69±0.22^{bc}	12.98±0.36^{ab}	12.83±0.15
Overall	13.63±0.18^{a}	11.85±0.16^{c}	12.91±0.15^{b}	13.18±0.15^{ab}	
Egg mass/hen (g)					
Period I	161.24±3.78^{ab}	156.66±7.58^{bB}	164.29±5.01^{abA}	176.11±5.72^{aA}	164.68±4.12^{A}
Period II	153.82±5.18^{b}	169.31±4.22^{aA}	132.81±8.01^{cB}	138.14±6.24^{bcB}	148.57±5.52^{B}
Overall	315.06±8.77^{ab}	320.27±7.81^{a}	297.11±6.18^{b}	314.25±8.24^{ab}	
Total mortality rate (%)	8.25±2.52	7.18±2.67	9.95±3.15	8.95±16	

^{a,b,c}Means in a row with no common superscript differ significantly ($p \leq 0.05$), ^{A,B}Means in a column with no common superscript differ significantly ($p \leq 0.05$), [1]Period I: After 3 weeks from starting of the treatment (42 weeks of age), [2]Period II: After 3 weeks from the first period (45 weeks of age), values are given in Mean±SE

Table 2: Effects of OM supplementation on egg quality traits

	Treatments				
Items	Control	0.5 mL	1 mL	2 mL	Overall
Egg Shape Index (ESI)					
Period I[1]	76.62±0.42	77.69±0.85	78.09±0.52	77.32±0.51	77.65±0.45
Period II[2]	76.92±0.62	79.38±0.74	78.66±0.66	77.58±0.72	77.59±0.51
Overall	76.68±0.39^{b}	78.68±0.45^{a}	78.21±0.41^{a}	77.36±0.45^{ab}	
Yolk Index (YI)					
Period I	43.73±0.36^{b}	50.22±042^{aA}	42.28±0.31^{b}	42.56±0.38^{b}	44.45±0.38
Period II	42.13±0.73^{b}	46.12±0.55^{aB}	42.87±0.73^{b}	42.81±0.38^{b}	43.61±0.64
Overall	43.41±0.33^{b}	47.83±0.36^{a}	42.41±0.52^{b}	42.61±0.37^{b}	
Internal Quality Unit (IQU)					
Period I	97.23±0.37^{a}	97.89±0.82^{a}	96.53±0.51^{a}	93.66±0.44^{b}	96.25±0.56
Period II	96.81±0.48^{b}	99.14±0.99^{a}	96.00±0.74^{b}	92.97±0.64^{c}	95.85±0.44
Overall	96.94±0.31^{b}	98.38±0.38^{a}	96.42±0.42^{b}	93.52±0.39^{c}	
Shell thickness (STH, µ)					
Period I	21.16±0.32^{a}	19.21±0.48^{b}	18.37±0.41^{b}	18.97±0.27^{b}	19.58±0.46
Period II	19.47±0.58	19.01±0.31	18.41±0.47	19.00±0.41	19.02±0.46
Overall	20.81±0.29^{a}	19.08±0.25^{b}	18.38±0.27^{c}	18.97±0.23^{bc}	

^{a,b,c}Means in a row with no common superscript differ significantly ($p \leq 0.05$). ^{A,B}Means in a column with no common superscript differ significantly ($p \leq 0.05$). [1]Period I: After 3 weeks from starting of the treatment (42 weeks of age). [2]Period II: After 3 weeks from the first period (45 weeks of age), values are given in Mean±SE

lowest values ($p \leq 0.05$) compared with other groups during the 1st, 2nd and entire experimental periods. Control group had significantly higher egg shell thickens than treated groups during the 1st and entire experimental periods.

Reproductive traits: Fertility, hatchability and embryonic mortality percentages: Fertility (F) and Hatchability (H) percentages as affected by treatments are shown in Table 3. Higher levels of OM (1 and 2 mL) had significantly ($p \leq 0.05$) decreased F and H% compared to lower level during the 1st, 2nd and entire experimental periods. However, eggs produced from birds supplemented with 0.5 mL showed significantly higher F an H% compared with control group during the 1st and 2nd periods. Furthermore, F and H% during the 2nd period were significantly improved in eggs produced from birds supplemented with 1 and 2 mL OM compared to the 1st period.

Eggs from 0.5 mL treated birds had lower Early Dead Embryo (EDE) and higher Late Dead Embryo (LDE) and Dead In Shell (DIS) percentages than the other treated groups ($p \leq 0.051$) during the 1st, 2nd and entire periods studied. Moreover, eggs produced from control groups had higher in the 1st period and lower in the 2nd period for the Piped Dead Embryos (PDE) than other treatment groups. Furthermore,

Table 3: Effect of OM supplementation on fertility, hatchability and embryonic mortality percentages

	Treatments				
Items	Control	0.5 mL	1 mL	2 mL	Overall
Fertility (%)					
Period I[1]	83.65±1.87^{b}	88.43±0.92^{a}	54.18±1.67^{cB}	49.52±3.15^{dB}	68.78±1.98^{B}
Period II[2]	81.41±1.26^{b}	89.11±0.23^{a}	81.08±2.67^{bA}	81.75±2.34^{bA}	83.38±1.64^{A}
Overall	82.53±1.64^{ab}	88.77±0.35^{a}	67.62±5.14^{b}	65.67±7.16^{b}	
Hatchability (%)					
Period I	62.83±2.35^{b}	68.48±1.04^{a}	39.64±2.47^{cB}	37.54±3.47^{cB}	51.86±1.56^{B}
Period II	64.31±2.64^{b}	67.51±1.15^{a}	47.41±2.14^{dA}	52.22±2.87^{cA}	57.84±1.64^{A}
Overall	63.57±1.67^{a}	68.24±0.48^{a}	43.53±2.61^{b}	44.86±4.12^{b}	
Early dead embryo (%)					
Period I	54.21±2.34^{cB}	42.37±1.92^{dB}	70.41±3.45^{b}	81.94±5.14^{aA}	61.82±2.35
Period II	69.65±2.64^{bA}	51.05±1.41^{dA}	74.61±2.36^{a}	54.00±1.06^{cB}	62.26±1.24
Overall	61.93±4.49^{a}	46.58±2.56^{b}	72.51±1.23^{a}	67.97±8.06^{a}	
Late dead embryo (%)					
Period I	33.88±1.36^{aA}	51.25±1.42^{aA}	27.38±2.14^{cA}	18.08±2.17^{dB}	32.52±1.15^{A}
Period II	21.83±2.34^{bB}	31.66±2.31^{aB}	16.07±1.67^{cB}	30.00±1.08^{aA}	24.68±1.34^{B}
Overall	27.85±3.49^{b}	41.34±5.25^{a}	21.73±3.29^{b}	24.02±3.44^{b}	
Dead in shell (%)					
Period I	7.61±0.38^{aA}	6.87±0.88^{aA}	2.19±1.85^{b}	0.00±0.00^{cB}	3.81±0.48^{B}
Period II	5.01±1.02^{bB}	9.16±1.17^{aB}	4.27±1.67^{b}	7.51±1.51^{aA}	6.54±0.59^{A}
Overall	6.31±0.87^{ab}	8.02±0.78^{a}	3.23±0.64^{a}	3.75±2.14^{a}	
Pipped (%)					
Period I	4.31±0.95^{a}	1.01±1.41^{bB}	00.0±00.0^{bB}	0.00±0.00^{bB}	1.35±0.24^{A}
Period II	3.51±0.85^{b}	7.66±0.94^{aA}	5.03±1.03^{bA}	8.51±0.51^{aA}	6.05±0.75^{B}
Overall	3.91±0.49	4.33±1.96	2.53±1.51	4.25±2.46	

^{a,b,c}Means in a row with no common superscript differ significantly ($p \leq 0.05$). ^{A,B}Means in a column with no common superscript differ significantly ($p \leq 0.05$), [1]Period I: After 3 weeks from starting of the treatment (42 weeks of age), [2]Period II: After 3 weeks from the first period (45 weeks of age), values are given in Mean±SE

Table 4: Effects of OM supplementation on reproductive organs of male and female quails at 6 weeks of experiment

	Treatments			
Items	Control	0.5 mL	1 mL	2 mL
Females				
Body weight (g)	266.60±12.5	286.00±13.6	283.80±16.8	278.00±9.4
Ovary weight (g)	7.91±0.51	8.93±0.71	8.29±0.49	8.89±1.05
Ovary weight (%)	2.96±0.14	3.11±0.13	2.94±0.17	2.89±0.32
Oviduct weight (g)	9.82±1.05	9.16±0.81	10.04±0.35	10.67±0.56
Oviduct weight (%)	3.65±0.31	3.19±0.35	3.61±0.32	3.84±0.16
Oviduct length (cm)	38.61±1.29	36.66±1.85	35.10±0.97	39.50±2.01
Average ovarian yellow follicle number	5.00±0.24^{ab}	5.67±0.32^{a}	3.81±0.37^{b}	3.61±0.41^{b}
Average ovarian yellow follicle size (mm)	13.86±0.65	14.01±0.35	13.87±0.94	14.40±0.58
Males				
Body weight (g)	230.80±12.9	242.60±14.2	231.40±12.2	206.60±7.6
Total testes weight (g)	7.65±0.83	6.76±0.52	6.57±0.62	6.77±0.81
Total testes (%)	3.29±0.23	2.81±0.12	2.82±0.14	3.26±0.31

^{a,b} Means in a row with no common superscript differ significantly ($p \leq 0.05$), values are given in Mean

LDE, DIS and pipped (%) during the 2nd period were significantly reduced in eggs produced from 2 mL group compared to the 1st period.

Reproductive organs: No remarkable effects due to treatments on reproductive organs of female and male quails were found throughout the experimental periods except in Ovarian Yellow Follicle Number (OYFN). Birds received 0.5 mL showed significantly higher OYFN than that in 1 and 2 mL groups and not significant with control group (Table 4).

Serum biochemical parameters: Results in Table 5 show the effects of OM supplementation on some serum biochemical parameters. Results showed no significant changes in serum total protein, albumin, urea and creatinine among experimental groups during 1st and 2nd periods compared with control group. However, birds received 0.5 mL showed significantly reduced serum concentrations of total lipids, triglycerides and cholesterol compared with other groups during 1st and 2nd periods. In contrast, serum concentrations of liver enzymes ALT and AST were significantly higher in

Table 5: Effect of OM supplementation on some serum biochemical analysis

	Treatments				
Parameters	Control	0.5 mL	1 mL	2 mL	Overall
Total protein (g dL^{-1})					
Period I[1]	4.50±0.59	4.38±1.92	4.67±0.60	4.40±0.82	4.55±0.51
Period II[2]	5.00±0.42	5.37±0.19	4.90±0.21	4.93±0.52	5.08±0.46
Overall	4.75±0.18	4.87±0.45	4.78±0.51	4.66±0.42	
Albumin (g dL^{-1})					
Period I	1.83±0.52	2.10±0.16	1.89±0.58	2.18±0.53	2.01±0.51
Period II	2.17±0.20	2.27±0.15	2.10±0.21	3.35±0.26	2.47±0.51
Overall	2.00±0.18	2.18±0.14	1.99±0.35	2.76±0.24	
Urea (mg dL^{-1})					
Period I	9.83±0.44	9.47±0.29	10.00±0.58	10.07±0.23	9.81±0.25
Period II	11.90±0.49	11.57±0.23	12.23±0.23	11.93±0.52	11.91±0.28
Overall	10.86±0.48	10.52±0.23	11.11±0.28	11.01±0.42	
Creatinine (mg dL^{-1})					
Period I	0.83±0.88	0.83±0.57	0.93±0.67	1.02±0.50	0.89±0.34
Period II	1.57±0.23	1.47±0.27	1.53±0.18	2.10±0.58	1.66±0.25
Overall	1.21±0.24	1.15±0.34	1.23±0.24	1.51±0.42	
Total lipids (mg dL^{-1})					
Period I	626.67±14.53^{a}	557.97±18.87^{b}	652.67±6.36^{a}	623.33±14.53^{a}	614.75±9.17
Period II	676.33±12.25^{a}	582.67±19.37^{b}	692.67±8.96^{a}	662.08±14.28^{a}	653.46±11.34
Overall	651.51±12.35^{a}	570.32±17.68^{b}	672.67±7.85^{a}	642.71±14.35^{a}	
Cholesterol (mg dL^{-1})					
Period I	236.33±4.91^{a}	205.00±2.89^{b}	244.83±2.89^{a}	245.00±5.00^{a}	232.68±3.00
Period II	248.33±4.41^{a}	215.33±2.60^{b}	251.67±1.67^{a}	251.67±6.00^{a}	241.67±4.12
Overall	242.33±4.12^{a}	210.16±1.25^{b}	248.23±2.15^{a}	248.33±4.56^{a}	
Triglycerides (mg dL^{-1})					
Period I	212.00±6.11^{a}	185.00±2.89^{b}	224.63±3.22^{a}	216.67±8.82^{a}	209.38±5.32
Period II	258.67±8.09^{a}	210.00±5.55^{b}	271.00±10.69^{a}	276.67±3.33^{a}	253.86±5.25
Overall	235.33±7.25^{a}	197.21±4.12^{b}	247.81±4.14^{a}	246.77±5.23^{a}	
ALT (IU L^{-1})					
Period I	41.00±2.08^{b}	42.67±2.96^{b}	64.67±5.17^{a}	66.67±8.82^{a}	53.38±2.15
Period II	44.00±2.31^{b}	46.00±3.06^{b}	64.67±7.57^{a}	65.30±5.09^{a}	54.85±2.64
Overall	42.51±2.14^{b}	44.33±2.95^{b}	64.67±5.64^{a}	65.98±5.34^{a}	
AST (IU L^{-1})					
Period I	101.33±1.85^{b}	95.50±2.93^{b}	130.00±2.03^{aB}	138.00±4.16^{a}	116.38±2.11
Period II	108.33±7.26^{b}	120.33±3.18^{b}	141.67±6.01^{aA}	155.33±2.60^{a}	131.54±3.16
Overall	104.83±5.14^{b}	107.91±2.35^{b}	135.83±5.15^{a}	146.66±3.25^{a}	

^{a,b}Means in a row with no common superscript differ significantly ($p \leq 0.05$), ^{A,B}Means in a column with no common superscript differ significantly ($p \leq 0.05$), [1]Period I: After 3 weeks from starting of the treatment (42 weeks of age), [2]Period II: After 3 weeks from the first period (45 weeks of age), values are given in Mean±SE

groups treated with 1 and 2 mL of OM than those recorded in 0.5 mL and control groups in both experimental periods. Furthermore, liver enzymes ALT and AST during the 2nd period were significantly increased in birds received 1 mL insignificantly increased in birds received 2 mL compared to the 1st period.

Histopathological changes in liver: Figure 2 shows disruption of the normal hepatic lobule architecture. In the livers of birds supplemented with both (C) 1 mL and (D) 2 mL of OM, the majority of hepatic cells, especially in the certrilobular and mid zonal areas, showed clear vacuoles within their cytoplasm. These vacuoles ranged from small multiple vacuoles to a single large one that peripheralized the nucleus. Birds treated with 2 mL OM showed multifocal areas of necrosis adjacent to the portal areas. Those areas were infiltrated with lymphocytes.

Correlation coefficients between increasing level of OM and some traits studied: As shown in Table 6, significant ($p<0.01$) negative correlations were found between concentrations of OM and fertility, hatchability, ovarian follicle number and a trend toward negative correlation (not statistically significant) with laying rate and internal quality unit. There were positive moderate correlations between OM levels and serum levels of liver enzymes (ALT and AST). Results indicted negative correlation were estimated between serum concentration of liver enzymes and

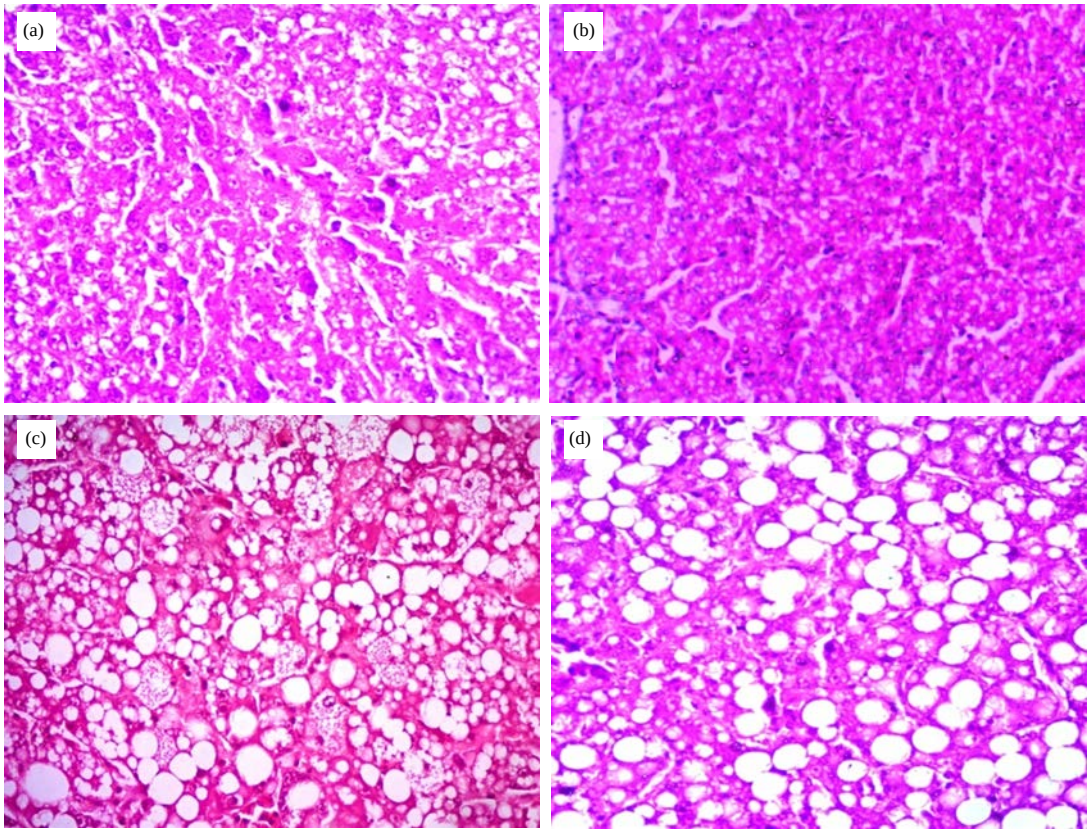

Fig. 2(a-d): Cross sections of livers of birds supplemented with (a) 0 mL, (b) 0.5 mL, (c) 1 mL and (d) 2 mL of OM

Table 6: Correlation coefficients between some traits studied

						Liver enzymes	
Traits	Laying rate	Fertility	Hatchability	Ovarian follicle No	Internal quality unit	ALT	AST
Oil mixture	-0.122	-0.768**	-0.800**	-0.498**	-0.335	0.203	0.337
Laying rate	1.000	0.579**	0.472*	0.558**	-0.023	-0.695**	-0.418*
Fertility		1.000	0.954**	0.729**	0.322	-0.404*	-0.129
Hatchability			1.000	0.722**	0.311	-0.332	-0.462
Ovarian follicle No			1.000	0.306	-0.539**	-0.349	

*p≤0.05, **p≤0.01, ALT: Aminotransferase, AST: Aspartate transferase

each of laying rate, fertility, hatchability (%), ovarian follicle numbers. Significant ($p<0.05$) positive correlations were observed between laying rate and fertility, hatchability and ovarian follicle number.

DISCUSSION

Usage of natural and herbal products such as OM at high levels is a popular concept that is believed to increase productive and reproductive performance of birds. Similarly, administration of high levels for long periods is believed to be more effective without considering the other adverse effects on the birds. Previous studies have showed some benefits such as immunomodulation, anti-inflammatory effect and others. Besides efficacy, these supplements also have to be safe to the animals, consumers of products from animals fed such products and the environment.

The efficacy of active components from natural compounds may depend on the dosage used. While no effects may be observed with small doses, large doses may even be toxic (Frankic *et al*., 2009). In our study, OM supplementation had affected the studied traits in two ways. The first one is improvement of most productive and physiological traits with a lower level of OM (0.5 mL L^{-1}) compared to the control group while the second one is a deterioration of the same traits with higher doses (1 or 2 mL L^{-1}) compared to the control group.

Positive results with low OM levels in this study are consistent with those reported by other researchers. Bozkurt *et al*. (2009) reported that broiler breeder hens given the lower level of OM (24 and 48 mg kg^{-1} diet from day one till 46 week of age) produced a higher number of eggs, fertility and hatchability percentages as compared with those of other treatments. Cabuk *et al*. (2014) found that OM had beneficial effects on egg production and feed conversion rate when used as a dietary supplement. Ozek *et al*. (2011) found that the hen-day egg production, egg shell thickness were significantly higher in brown layers when given diets supplemented with an OM compared to the control, between 54-74 weeks of age.

Possible explanations of the beneficial effects of OM (0.5 mL) on most of the physiological performance is probably the antioxidant activity of the active ingredients included within the oil blend which has been reported for different Essential Oils (EOs), including lavender (*Lavandula* spp) (Lee and Shibamoto, 2002), eucalyptus (*Eucalyptus oblique*) (Lee and Shibamoto, 2001) and peppermint (*Mentha piperita*). The antioxidant mechanisms of EOs is based on both their ability to donate a hydrogen or an electron to free radicals and their ability to delocalize the unpaired electron within the aromatic structure (Fernandez-Panchon *et al*., 2008) and protecting other biological molecules against oxidation (Giannenas *et al*., 2013). This antioxidant activity could reflect on the normal architecture of the liver cells and liver enzymes (ALT and AST) which showed significant decrease. The beneficial effect was also noticed on the hypolypidemic action as the levels of total lipids, triglycerides and cholesterol in serum in the group supplemented with OM at the level of 0.5 mL. Reduced cholesterol could be attributed to the effect of some active components within the OM such as anthenol, lemonin and sesame. The anethole is considered as a phytoestrogen which can regulate serum cholesterol levels in the human and animals (Christaki *et al*., 2011). The hypocholesterolemic effect of lemongrass oil is due to the inhibition of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, which is a key regulatory enzyme in cholesterol synthesis (Crowell, 1999). Wu (2007) reported that sesame contains phytosterols that are associated with reduced levels of blood cholesterol by regulating the transcription process of hepatic metabolizing enzymes for lipids (Kiso, 2004) and increase in the activity of various hepatic enzymes involved in fatty acids oxidation (Arachchige *et al*., 2006) thereby reducing serum and liver lipids (Lim *et al*., 2007). The antioxidant nature of medicinal plants been suggested to alleviate the negative influence of environmental stressors and improve immune function to combat different types of diseases (Al-Kassie *et al*., 2011). In contrast, increasing levels of OM (1 and 2 mL L^{-1}) caused significantly reduced performance in most studied traits compared to control group. It was noticed that OM given at levels of 1 and 2 mL for 6 weeks caused damage to the liver. This damage paralleled with increase in liver enzymes, ALT and AST, which are usually considered as indicators of dysfunction or hepatocellular damage. Increases in ALT and AST levels are thought to be due to physiological stresses on liver which plays a major role in all productive and reproductive traits studies explaining the adverse effects of high levels of OM. Also, the reduced quail performance with high levels OM could be due to one or more of the active components of the mixture, such as cineole component which could have inflammatory properties.

The non-significant changes in total protein and albumin may be due to the presence of monounsaturated and polyunsaturated fatty acids in the OM (Choi *et al*., 2008). These unsaturated fatty acids are required for the synthesis of immune regulating substances (Hwang, 2000). It could be attributed to that the OM is not stimulated the synthesis of total protein and albumin in the liver. Serum protein levels are regulated via synthesis in the liver and its levels thus reflect the synthetic ability of the liver (Okokon *et al*., 2010) so that administration of high levels of OM could have an inhibitory action on the hepatic cells for production of total proteins and albumin as such as hepatic toxicity. Detrimental effects of high levels (1 or 2 mL) for long period of time coincided with pathological findings in liver.

The effects of herbal plant extracts on blood lipid profiles have been controversial. Hyperlipidemic effects were seen with some medicinal plants (Toghyani *et al*., 2010) whereas hypolipidemia was reported by others (Lee *et al*., 2004). The discrepancies between studies might be attributed to the differences in herbal plant used, levels, form of plant, route of administration as well as experimental conditions. The levels of urea and creatinine in the treated groups did not show any significant difference with respect to the control values, an indication that OM is not nephrotoxic at the levels used in this experiment.

In general, our results indicated that different levels of OM affected birds in different ways. High levels used had negative effects on physiological, reproductive performance and pathological findings in liver. The dose of OM at 0.5 mL L^{-1} of drinking water may safe and enough to improve the performance and health of laying Japanese quail.

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