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EFFECT OF DIETARY SUPPLEMENTATION WITH AN ESSENTIAL OILS BLEND AND MANNAN OLIGOSACCHARIDE ON: 2-THE REPRODUCTIVE PERFORMANCE, EGG QUALITY AND BLOOD PARAMETERS OF GOLDEN MONTAZAH LAYERS AT LATE PHASE OF EGG PRODUCTION

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SUMMARY

The experimental work of the present study was carried out at El-Takamoly Poultry project (TPP), Fayoum Governorate, Egypt, during the period from March to July 2014. This study was undertaken to assess the dietary effects of essential oil blend (EOB), mannan oligosaccharides (MOS) and their combination on the reproductive performance, egg quality and blood parameters of Golden Montazah laying hens at late phase of egg production. A total number of 720 laying hens plus 72 rooster of Golden Montazah at 56 weeks of age were allocated randomly into six treatments groups (120 hens plus 12 rooster), each group was equally subdivided into three replicates (40 hens plus four rooster). Birds were distributed into 18 pens (40 hens and four rooster each) in such order to have a similar mean body weight and average daily egg production. The dietary treatments used in this study were as follows: 1-Birds were fed the control diet (diet 1). 2- diet 1 + 200 mg prepared essential oil blend (PEO)/kg diet. 3-diet 1 + 100 mg commercial essential oil blend (CEO)/kg diet. 4-diet 1 + 500 mg MOS/kg diet. 5-diet 1+ 200 mg PEO + 500 mg MOS/kg diet. 6-diet 1 + 100 mg CEO + 500 mg MOS/kg diet. Results obtained could be summarized in the following: There were insignificant differences among all dietary treatments in Eq at 64 and 72 weeks, fertility and hatchability% at 64, 68 and 72 weeks. There were insignificant differences among all dietary treatments in chemical composition of eggs, except, cholesterol concentration in the yolk at 72 weeks. Laying hens fed diet containing 100 mg CEO +500 mg MOS/kg diet had significantly lower cholesterol in the yolk, whereas, those fed control diet had significantly higher cholesterol in the yolk at 72 weeks. No significant differences due to EOB and MOS supplementation on biochemical plasma parameters, except, total protein, globulin and calcium which were significantly affected, laying hens fed diet containing 100 mg CEO +500 mg MOS/kg diet had significant higher values of total protein, globulin and calcium, whereas, those fed control diet had significant lower values of these parameters. Neither EOB nor MOS had any significant effect on hematological parameters. Laying hens fed diet supplemented with 100 mg CEO +500 mg MOS/kg diet had significant higher values of HDL cholesterol, total antioxidant capacity and GSH-Px activity in plasma. While, those fed control diet had higher values of total lipid, triglycerides and LDL cholesterol in plasma. Laying hens fed diet supplemented with 100 mg CEO +500 mg MOS/kg diet had significant higher value of immune response to antibody titer of Avian Influenza virus (AIV). While, those fed control diet had significant lower value of AIV. No significant differences of Newcastle Disease Virus among treatment groups. In conclusion, the results of this study indicated that feeding Golden Montazah laying hens during the period from 56 to 72 weeks of age on diets containing 100 mg CEO +500 mg MOS/kg diet improved the reproductive performance and increased stimulation of immune, HDL cholesterol, GSH-Px activity, total antioxidants activity in plasma and decreased cholesterol concentration in the egg yolk. This may lead to produce enriched eggs that are healthier for human consumption especially for those suffering from heart diseases.

Keywords: *essential oils, mannan oligosaccharides, reproductive performance, egg quality and immune response.*

INTRODUCTION

Poultry production in Egypt has become one of the biggest agriculture industries and its improvement is one of the main objectives of both private and public sectors. Many attempts in the field of animal

nutrition are being done to achieve an increase in animal production and thereby profit. Feed additives are important materials that can improve the efficiency of feed utilization and poultry performance. Phytogens are relatively young group of additives which, in recent few years, drew attention of the feed producing industry (Hashemi and Davoodi, 2010).

Since banning antibiotic growth promoters in animal feeds. So, there are a number of non-therapeutic alternatives to antibiotic growth promoters, including enzymes, organic acids, probiotics, prebiotics, herbs, immune stimulants and specific management practices or plant extract (Hernandez *et al.,* 2004; Griggs and Jacob, 2005; Awadein *et al.,* 2010 and Uddin, 2014).

Compared to synthetically derived antibiotics and inorganic chemical substances, these products originated from plants are natural, proven less toxic, does not produce residues, could became an ideal feed additives and successfully replace antibiotic growth promoters in feed (Hernandez *et al.,* 2004 and Uddin, 2014). Heinrichs *et al.* (2003) hypothesized that the antibiotics that have been added to animal feed, have resulted in human pathogens' resistance to specific antibiotics. On the other hand, using alternatives to antibiotic growth promotants in commercial chickens have become important mainly because of apprehensions about the possible development of resistant bacteria. One of many such classes of alternatives is the prebiotic mannan oligosaccharide (MOS). Mannan oligosaccharides is complex sugars and derived from the outer cell wall of either *Saccharomyces cerevisiae* or *Saccharomyces boulardii* yeasts (Hofacre *et al.,* 2003). Mannose, the main component of MOS, is a unique sugar because many enteric bacteria have receptors that bind to it (Griggs and Jacob, 2005).

Essential oils (EOs) are very complex mixtures of compounds and their chemical compositions and concentrations are variable. An EOs is a mixture of fragrant, volatile compounds, named after the aromatic characteristics of plant materials from which they can be isolated (Oyen and Dung, 1999). Essential oils are natural products that exhibit various biological properties, such as analgesic, anticonvulsant, and anxiolytic effects, those effects are attributed to the monoterpenes, which are the major chemical components of these EOs. Phenolic compounds commonly found in EOs contribute greatly to the antibacterial and antioxidant activity.

Bakkali *et al.* (2008) reported that in eukaryotic cells, EOs can have pro-oxidant effects on inner cell membranes and organelles causing concentration and type dependent cytotoxic effects on living cells without genotoxicity. Craig (1999) reviewed the role of herbs and their EOs as to their cholesterol lowering properties and in the protection against cancer. Hood *et al.* (1978) tested the hypothesis that dietary EOs may inhibit biosynthesis of farnesyl pyrophosphate, a precursor of cholesterol synthesis.

Several scientific studies have examined the antioxidant properties of some selected EOs as natural antioxidant feed additives. However, in spite of this, there is hardly any information comparing EOs and MOS. Therefore, this study aim to assess the dietary effects of prepared essential oil blend (PEO) or commercial essential oil blend (CEO) and MOS on the reproductive performance, egg quality and blood parameters of Golden Montazah laying hens at late egg production period.

MATERIALS AND METHODS:

The experimental work of the present study was carried out at El-Takamoly Poultry project (TPP), Fayoum Governorate, Egypt, during the period from March to July 2014. This study was undertaken to assess the dietary effects of EOB, MOS and their combination on the reproductive performance of Golden Montazah laying hens at late egg production period, as well as on the age related oxidative stress, metabolic profile and immune status. The chemical analyses were performed in the Laboratories of the Animal Production research institute, agriculture research center, ministry of Agriculture, according to the procedures outlined by A.O.A.C. (1990).

At 54 week of age, experimental hens were selected from the Golden Montazah stock reared at TPP on the basis of their egg production (EP) and body weight (BW) and assigned to feeding regimens for two weeks, prior to trail to ensure that the EP and BW profile in each group was similar and to adjust the daily feed amount for all birds (there were no significant differences between replicates). Then, the experiment started at the age of 56 weeks and ended at the age of 72 weeks.

A total number of 720 laying hens plus 72 roosters of Golden Montazah at 56 weeks of age were allocated randomly into six treatments groups (120 hens plus 12 roosters), each group was equally subdivided into three replicates (40 hens plus four rooster). Birds were distributed into 18 floor pens (40 hens and four roosters each) in such order to have a similar mean BW and average daily EP.

All birds were reared under the same management conditions (open system), in similar open-sided floor pens with (3×2.5 m; 6 birds/m^2). Each pen was equipped with two circular hanging feeders, two hanging drinkers and one 10-hole nest box. The applied drinking system had the automatic bell type and the handy feeder pan system was performed. The floor of pens was bedded with wheat straw as litter material. With the exception of hand feeding, the housing condition was comparable with commercial standards. Extra artificial light source was used, 100 watt lamps with 3m distance from each other and in 2.30 m height from the ground were considered for lighting giving a total of 16-hour day photoperiod (16L :8D), throughout the experimental period (16 weeks). The minimum and maximum average of ambient temperatures was 26±1˚C and 34±1˚C with 63±2.5% relative humidity. All hens were fed with experimental diets *ad libitum* from 56 to 72 weeks.

Tested Materials:

1-Prepared essential oil blend (equal mixture of cinnamon and thyme extracted oils) purchased from squeeze and extraction medicinal and aromatic oils unit, National Research center, Egypt.

2-Commercial essential oil blend (Enviva EO ®, Danisco Animal Nutrition, Marlborough, UK; active ingredients were cinnamaldehyde and thymol). The commercial product was a mixture of 2 different EOs (cinnamon and thyme) derived from selected herbs, purchased from Multi vita Animal Nutrition company.

3-Mannan oligosaccharide (Bio-Mos®; Alltech, Inc., Nicholasville, KY, USA). The commercial product Bio-Mos contains MOS, yeast cell wall derivate of *saccharomyces cerevisiae* purchased from International Free Trade Corporation.

A corn-soybean mash meal as a basal experimental layer diets were formulated to satisfy nutrient requirements (iso-nitrogenous and caloric) of Golden Montazah laying hens (16% CP and 2750 Kcal ME/Kg diet) according to the Egyptian Agriculture Ministry Decree No 1498 (1996) issued by Ministry of Agriculture. The composition and calculated analyses of the control diet are shown in Table (1).

Table (1): Composition and calculated analyses of the control diet:

Ingredient	%
Yellow corn, ground	65.32
Soybean meal (44% CP)	24.20
Wheat bran	1.10
Calcium carbonate	7.10
Mono-calcium phosphate	1.50
Sodium chloride	0.30
Vit. and Min. premix*	0.40
DL-Methionine	0.03
Na_2So_4	0.05
Total	100
Calculated analyses% (according to NRC, 1994):	
Crude protein	16.06
Ether extract	2.71
Crude fiber	3.32
Available phosphorus	0.48
Calcium	3.02
Lysine	0.87
Methionine	0.32
Methionine + Cysteine	0.58
ME, Kcal./kg	2759.60

* *Each 4.0 kg of premix supplies one ton of the diet with: Vit. A, 15000000 I.U; Vit. D_3, 3300000 I.U; Vit. E, 60000 mg; Vit. K_3, 3600 mg; Vit. B_1, 2200 mg; Vit. B_2,12000 mg; Vit. B_6, 5500 mg; Vit. B_{12}, 20 mg; biotin, 250 mg; folic acid, 1500 mg; pantothenic acid, 15000 mg; Zn, 80000 mg; Mn, 100000 mg; Fe, 80000 mg; Cu, 9000 mg; I, 1100 mg; Co, 200 mg; Se, 300 mg; Choline, 600000 mg and extra F T B, 60000 enzyme unit, and complete to 4.0 Kg by calcium carbonate (produced by Multi vita co., Egypt under authority of Adisseo co., France, Reg. No, 6589).*

The dietary treatments used in this study were as follows:

1-Birds were fed the control diet (diet 1). 2-diet 1 + 200 mg PEO/kg diet.

3-diet 1 + 100 mg CEO/kg diet. 4-diet 1 + 500 mg MOS/kg diet.

5-diet 1 + 200 mg PEO + 500 mg MOS/kg diet.

6-diet 1 + 100 mg CEO + 500 mg MOS/kg diet.

Total number of 72 eggs (12 eggs from each treatment) were randomly collected twice (at the end of 64 and 72 weeks of age) and individually weighed to the nearest 0.1g and broken onto a flat surface where the height of the albumen to the nearest 0.1 mm was measured half way between the yolk and the edge of the inner thick albumen by using an electronic albumen height gauge. The yolk was separated from the albumen and weighed and, yolk visual color score was determined by matching the yolk with one of the 15 bands of the "1961, Roche Improved Yolk Color Fan". The same person performed all of the yolk color determinations. The shells were dried at room temperature for 3 days and weighed; egg shell thickness (including shell membranes) was measured using a micrometer at three locations on the egg (air cell, equator, and sharp end). The weight of the albumen was calculated as the difference between the weight of the egg and the weight of the yolk and shell. Haugh unit score was applied from a special chart using egg weight and albumen height which was measured by using a micrometer according to Haugh (1937). Egg shape index% (Carter, 1968) and yolk index% (Well, 1968) were calculated.

At 64 and 72 weeks of age, chemical analyses of egg component was performed in duplicate samples for either yolk or albumen to determine percentages of DM, CP (N X 6.25), EE and ash according to the methods of A.O.A.C. (1990), nitrogen free extract (NFE) was calculated by difference. At the same ages 4 eggs were taken randomly from each pen to determine yolk cholesterol.

Fifty settable eggs per pen were set for incubation biweekly (64, 68 and 72 weeks of age). Eggs were incubated in automatic incubator at 37.8˚C dry bulb temperatures and 70% relative humidity, eggs turning every one hour (0-18 days). Eggs were candled on day 10 of incubation for monitoring infertile eggs, all infertile eggs were opened and examined for evidence of embryonic mortality, then all unhatched eggs were analyzed for developmental stage of dead embryos. The time of embryonic death was assigned to one of two categories, early dead (≤10 days) when blood islet or very small embryo with very large yolk sac was observed, late dead (11-21 days) when medium sized or fully formed embryo was observed. Fertility was expressed as the rate of fertile eggs to total eggs set. On day 19, eggs were transferred to baskets which were placed randomly into the hatcher cabinets at 36.8˚C dry bulb temperatures and 85% relative humidity. The number of eggs that hatched was recorded at 21.5 days of incubation. Within 2 hours after hatching, each chick was weighed, the body weights of chicks were determined using mean of individual chick weight (g).

At the end of experiment (72 weeks of age), six birds from each pen were taken around the average body weight was closest to the replicate mean. Two triplicate blood samples were collected from brachial vein, one into a heparinized test tubes for blood hematological parameters, and the other into a non-heparinized test tubes for biochemical parameters, and then plasma was separated by centrifuging at 3500 rpm for 15 minutes. The clear plasma samples were carefully drawn and transferred to dry, clean, small glass bottles and stored at -20^{o}C in a deep freezer until the time of chemical determinations. Fresh blood samples were taken to determine hemoglobin (Hg), hematocrit (Ht), total count of red blood cells (RBCs), total count of white blood cells (WBCs) and their differentiations (Heterophils% (H), lymphocytes% (L), and H/L ratio) according to Clark *et al.* (2009). The biochemical characteristics of blood were calorimetrically determined using commercial kits (Biodiagnostic Company Cairo, Egypt). Estimation of glutathione peroxidase (GSH-Px) activity (in whole blood) and total antioxidant capacity (in plasma, TAOC) using biodiagnostic kits.

Plasma samples was used to determine total protein(g/dl), albumin(g/dl), globulin (estimated by subtracting the values of albumin from the corresponding values of total protein), total lipids (mg/dl), cholesterol (mg/dl), low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), triglyceride, ALT, AST and calcium. All determinations were measured calorimetrically on spectrophotometer by using the suitable commercial kits (Biodiagnostic Company Cairo, Egypt).

Also, at 72 weeks of age, 3 hens from each pen were used, each hen was intradermally injected in the toe web of the left foot (into the webs between the second and the third digits) with 5 μg phytohemagglutinin-P (PHA-P) in 0.1 ml of sterile saline and measured with micrometer before injection and at 24, 48 and 72 hr after PHA-P injection. The toe web swelling was calculated as the difference between the thickness of the toe web before and after injection. At the end of experiment, hemagglutination- inhibition (HI) test was applied for determination of antibodies response in plasma samples according to OIE Manual (2005). Commercial enzyme-linked immunosorbant assay (ELISA) kits were used for detection of antibodies against nucleoprotein and matrix against of Newcastle Disease Virus (NDV) and against of Avian Influenza Disease Virus (AIDV). Hemagglutination-inhibition test

titer regarded as positive if there is inhibition at serum dilution of 1/16 (4 log2) using the ELISA technique.

Statistical analysis of results was performed using the General Linear Models (GLM) procedure of the SPSS software (SPSS, 2007), according to the follow general model: $Y_{ij}= \mu+T_i+e_{ij}$

Where: Y_{ij}: observed value

μ: overall mean

T_i: treatment effect (i: (1 to 6).

e_{ij}: random error

Treatment means indicating significant differences ($P\leq0.01$ and $P\leq0.05$) were tested using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Egg quality (Eq): Effects of dietary supplementation with an EOB and MOS on Eq of Golden Montazah laying hens at late phase of egg production are shown in Table 2. Dietary EOB and MOS effect were insignificantly affected for Eq at 64 and 72 weeks of age (Table 2). Numerically, laying hens fed diet containing 100 mg CEO +500 mg MOS/kg diet had higher values of shell% and shell thickness at 64 and 72 weeks of age, while, laying hens fed control diet had lower ($P>0.05$) values of albumen%, shape index%, shell% and shell thickness at 64 and 72 weeks of age (Table 2). From our results, numerically, it can be observed that egg quality is reduced (increase in albumen%), as the hen age increase.

In this regard, Shashidhara and Devegowda (2003); Hassan and Ragab (2007) and (Gurbuz *et al.*, 2011) reported that the MOS improved egg shell%, yolk% and yolk index and decreased eggshell deformation in layer hens (Cabuk *et al.,* 2006). Consistent with those reports, Bozkurt *et al.* (2012) found that both MOS and EOs increased eggshell weight, eggshell thickness and shell breaking strength, while they decreased relative albumen weight and albumen height compared with no added procedure. The better results obtained for the eggshell quality parameters in hens fed MOS could be due to the prebiotic influence on the metabolic activity of the beneficial bacteria colony within the layers' intestine, which positively influences mineral absorption rate, especially those of Ca^{2+} and Mg^{2+} (Roberfroid, 2000). Failures in terms of improving albumen and egg yolk were reported by other researchers when hens were fed on diets with EOs of thyme and rosemary (Bolukbasi *et al.,* 2008).

Chemical composition of eggs and cholesterol concentration in the yolk: Effects of dietary supplementation with an EOB and MOS on chemical composition of eggs and cholesterol concentration in the yolk of Golden Montazah laying hens at late phase of egg production are shown in Table 3. There were insignificant differences among all dietary treatments in chemical composition of eggs and cholesterol concentration in the yolk at 64 weeks of age except, NFE%. Laying hens fed diet containing 100 mg CEO/kg diet had higher NFE% at 64 weeks, whereas, those fed control diet +100 mg PEO/kg diet had lower NFE% at the same age. Except, cholesterol concentration in the yolk, there were insignificant differences among all dietary treatments in chemical composition of eggs at 72 weeks of age. Laying hens fed diet containing 100 mg CEO +500 mg MOS/kg diet had significantly ($P\leq0.01$) lower cholesterol (consequently lower fat, ($P>0.05$)), whereas, those fed control diet had significantly ($P\leq0.01$) higher cholesterol (higher fat, ($P>0.05$)) at 72 weeks (Table 3). In this respect, the decrease of fat% ($P>0.05$) and cholesterol in yolk egg was found to be correlated to decreasing these parameters in plasma (Table 6), these finding may be due to the effect of EOs components present in herbs on lipid metabolism. From these results, it could be concluded that EOB and MOS may have lowering effect total cholesterol in the yolk. This may lead to produce enriched eggs that are healthier for human consumption and useful for those suffering from heart diseases.

Table (2): Effects of dietary supplementation with an essential oil blend and mannan oligosaccharide on egg quality traits of Golden Montaza layers at late phase of egg production.

Item	Albumen%	Haugh unit	Yolk%	Yolk index%	Yolk color	Shape index%	Shell%	Shell thickness, mm
Treatments				At 64 week of age				
Control diet (D_1)	55.33	73.99	33.80	43.18	8.56	74.52	10.01	37.48
D_1+ 200 mg/kg diet PEO[1] (D_2)	56.70	75.36	32.73	41.84	9.00	76.11	10.27	37.96
D_1+ 100 mg/kg diet CEO[2] (D_3)	56.52	73.69	33.47	41.38	8.78	76.30	10.29	38.46
D_1+ 500 mg/kg diet MOS[3]	57.34	75.36	32.09	43.43	8.56	77.23	10.57	38.53
D_2+ 500 mg/kg diet MOS	57.65	74.91	32.06	41.36	8.33	75.55	10.57	38.74
D_3+ 500 mg/kg diet MOS	56.30	75.27	33.43	42.21	8.67	75.03	10.87	39.30
±SEM[4]	1.34	2.11	1.20	1.22	0.34	1.15	0.35	1.02
P value	0.863	0.986	0.851	0.753	0.815	0.617	0.604	0.862
				At 72 week of age				
Control diet (D_1)	57.31	71.03	32.71	40.27	7.67	74.11	9.98	36.02
D_1+ 200 mg/kg diet PEO (D_2)	58.88	72.78	30.63	39.91	8.44	76.35	10.21	37.35
D_1+ 100 mg/kg diet CEO (D_3)	57.42	73.44	32.37	40.75	7.89	75.62	10.22	38.00
D_1+ 500 mg/kg diet MOS	56.79	72.48	32.99	38.66	7.56	75.91	10.49	37.35
D_2+ 500 mg/kg diet MOS	56.92	73.38	32.41	39.71	8.00	76.40	10.49	38.34
D_3+ 500 mg/kg diet MOS	58.77	71.73	30.74	39.51	8.33	76.36	10.67	38.54
±SEM	0.99	2.45	0.90	1.03	0.30	1.11	0.34	1.14
P value	0.516	0.979	0.279	0.787	0.249	0.681	0.757	0.666

[1] *Prepared essential oil.* [2] *Commercial essential oil blend.* [3] *mannan oligosaccharide.* [4] *Pooled SEM.*

Table (3): Effects of dietary supplementation with an essential oil blend and mannan oligosaccharide on chemical composition of eggs of Golden Montaza layers at late phase of egg production.

Item	Moisture%	Protein%	Fat%	Ash%	NFE%	Yolk cholesterol
Treatments			At 64 week of age			
Control diet (D_1)	73.67	12.95	11.36	0.602	1.424^{bc}	16.13
D_1+ 200 mg/kg diet PEO[1] (D_2)	74.50	12.75	10.99	0.568	1.195^{c}	15.75
D_1+ 100 mg/kg diet CEO[2] (D_3)	73.77	12.76	10.68	0.608	2.192^{a}	15.08
D_1+ 500 mg/kg diet MOS[3]	73.47	12.76	11.20	0.634	1.936^{ab}	15.20
D_2+ 500 mg/kg diet MOS	73.70	12.83	10.70	0.618	2.158^{a}	15.63
D_3+ 500 mg/kg diet MOS	74.45	12.77	10.27	0.583	1.929^{ab}	15.23
±SEM[4]	0.40	0.10	0.39	0.02	0.22	0.38
P value	0.31	0.66	0.40	0.13	0.01	0.339
			At 72 week of age			
Control diet (D_1)	74.10	12.82	11.92	0.635	0.528	17.32^{a}
D_1+ 200 mg/kg diet PEO (D_2)	73.86	12.95	11.56	0.640	1.001	17.02^{a}
D_1+ 100 mg/kg diet CEO (D_3)	73.53	13.26	11.38	0.634	1.202	15.56^{b}
D_1+ 500 mg/kg diet MOS	73.01	13.74	11.82	0.641	0.797	14.94^{bc}
D_2+ 500 mg/kg diet MOS	74.10	12.85	11.31	0.627	1.115	15.41^{b}
D_3+ 500 mg/kg diet MOS	73.86	12.88	11.16	0.629	1.462	13.94c
±SEM	0.39	0.35	0.23	0.004	0.229	0.40
P value	0.355	0.384	0.165	0.070	0.094	0.00

[1] Prepared essential oil. [2] Commercial essential oil blend. [3] mannan oligosaccharide. [4] Pooled SEM.

[a–c] Means in a column with different superscripts differ significantly (P≤0.05).

In this regard, Azeke and Ekpo (2008) and Abdo *et al.* (2010) reported that the reduction in egg yolk cholesterol may be due to antioxidant activity and high catechin may have an inhibitory effect on intestinal absorption of lipid. The pure components of EOs inhibit hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductive activity (Crowell, 1999) which is a key regulatory enzyme in cholesterol synthesis and consequently the hypocholeterolemic effect (Lee *et al.,* 2004). These results agree with those reported by Ali *et al.* (2007) who found that addition of 0.25% thyme to laying hens diets significantly decreased the yolk total lipid, cholesterol and LDL compared with control group. Also, these results are in harmony with those obtained by Radwan *et al.* (2008) who found that addition of 1.0% thyme, rosemary or curcuma longa as natural antioxidants, in layer diets (El-Salaam strain) significantly decreased yolk total lipid.

Fertility and hatchability%: Effects of dietary supplementation with an EOB and MOS on reproductive traits of Golden Montazah laying hens at late phase of egg production are shown in Table 4. There were insignificant differences among all dietary treatments in reproductive traits of eggs at 64, 68 and 72 weeks of age. Numerically, laying hens fed diet containing 100 mg CEO +500 mg MOS/kg diet had higher values ($P>0.05$) of healthy chicks, chicks weight and hatchability% (lower early and late embryonic mortality%) at 64 and 68 weeks, whereas, those fed control diet had lower values of healthy chicks, chicks weight, fertility and hatchability% (higher abnormal chicks%) at the same ages (Table 4).

Laying hens fed diet containing 100 mg CEO +500 mg MOS/kg diet had insignificant higher values of healthy chicks, fertility and hatchability% (lower early and late embryonic mortality%) at 72 weeks (this may be due to lower fat and cholesterol concentration in the yolk). Whereas, those fed control diet had insignificant lower values of healthy chicks, fertility and hatchability% (higher early, late embryonic mortality and abnormal chicks%) at the same age(Table 4). So, the data indicated that dietary EO and MOS caused an improvement of fertility. From our results, numerically, it can be observed that healthy chicks, fertility and hatchability% are reduced (increase early embryonic mortality% and abnormal chicks%), as the hen age increase. This result is agreed in general with the results of Alsobayel (1992) who reported that hen age has an influence on the fertility of eggs.

In this respect, Speake *et al.* (1998) reported the fact that chick embryo development is associated with an accumulation of polyunsaturated fatty acids in tissue lipids. This making them susceptible to lipid peroxidation (Surai, 1999). There is evidence that the antioxidant components of herbs such thymol present in thyme can be transferred to eggs and deposited into yolk (Botsoglou *et al.,* 1997 and Galobart *et al.,* 2001) and increase the adaptation mechanism to deal with over production of free radicals, consequently increase hatchability and thymol can act as antioxidant in egg when introduced into the diets (Botsoglou *et al.,* 1997). Chicks from broiler breeders where both the breeder hens and roosters were fed MOS had a better innate immunity than control chicks (Shashidhara and Devagowda, 2003).

Dibner and Richards (2005) found that microflora also exert some undesirable effects such as toxin synthesis; high rates of gut epithelium turnover and competing with host for nutrients. Mucus was found to protect the intestines against microflora, enteropathogenic activity and normal digestive processes in the gut. So, mucus secreting cell development reportedly increased in the embryo at day 17 pre-hatch (Smirnov *et al.,* 2006).

***Blood parameters**:*

a-Biochemical plasma parameters: Impact of EOB and MOS supplementation on biochemical plasma parameters of Golden Montazah laying hens at late phase of egg production are shown in Table 5. The results indicated that no significant differences due to EOB and MOS supplementation on biochemical plasma parameters, except, total protein, globulin and calcium which were significantly ($P\leq0.01$ and $P\leq0.05$) affected. Laying hens fed diet containing 100 mg CEO +500 mg MOS/kg diet had significant higher values of total protein, globulin and calcium, whereas, those fed control diet had significant lower values of these parameters (Table 5). Similar results were previously observed by Hassan and Ragab (2007) who reported that addition of 0.1% MOS in diets of laying hens caused significant increases in serum total protein. Also, Ali *et al.* (2007) found that addition of thyme (as a natural antioxidant) numerically increased plasma calcium.

Table (4): Effects of dietary supplementation with an essential oil blend and mannan oligosaccharide on reproductive traits of Golden Montaza layers at late phase of egg production.

Item	Early embryonic mortality%	late embryonic mortality%	Abnormal chicks%	Healthy chicks%	Chick weight(g)	Fertility%	Hatchability%
Treatments				At 64 week of age			
Control diet (D_1)	7.71	16.98	2.03	73.28	34.57	89.49	75.32
D_1+ 200 mg/kg diet PEO[1] (D_2)	7.77	16.76	1.99	73.49	34.91	90.09	75.48
D_1+ 100 mg/kg diet CEO[2] (D_3)	7.40	15.30	1.83	75.47	35.03	90.12	77.30
D_1+ 500 mg/kg diet MOS[3]	7.45	16.19	1.73	74.63	34.67	90.54	76.36
D_2+ 500 mg/kg diet MOS	7.25	14.91	1.49	76.34	34.94	90.70	77.84
D_3+ 500 mg/kg diet MOS	7.21	14.16	1.53	77.11	35.58	90.29	78.63
±SEM[4]	1.10	3.18	0.77	3.11	0.28	1.10	3.58
P value	0.999	0.985	0.993	0.935	0.224	0.977	0.980
				At 68 week of age			
Control diet (D_1)	9.95	18.70	2.54	68.81	35.05	88.01	71.35
D_1+ 200 mg/kg diet PEO (D_2)	9.59	18.77	2.06	69.58	35.40	88.52	71.65
D_1+ 100 mg/kg diet CEO (D_3)	9.55	17.92	1.99	70.54	35.52	88.66	72.53
D_1+ 500 mg/kg diet MOS	9.52	18.69	2.01	69.78	35.16	89.85	71.78
D_2+ 500 mg/kg diet MOS	9.08	17.33	1.77	71.82	35.43	89.42	73.59
D_3+ 500 mg/kg diet MOS	9.02	16.53	1.78	72.68	36.08	89.24	74.46
±SEM	1.67	3.39	0.70	1.27	0.28	1.71	3.00
P value	0.999	0.995	0.972	0.947	0.224	0.975	0.970
				At 72 week of age			
Control diet (D_1)	14.45	17.13	3.19	65.23	36.40	87.10	68.42
D_1+ 200 mg/kg diet PEO (D_2)	14.19	16.21	2.57	67.03	36.09	87.38	69.60
D_1+ 100 mg/kg diet CEO (D_3)	13.23	16.19	2.31	68.27	36.01	87.36	70.58
D_1+ 500 mg/kg diet MOS	13.93	16.46	2.54	67.07	36.33	88.52	69.61
D_2+ 500 mg/kg diet MOS	12.52	15.69	2.04	69.75	36.51	88.32	71.79
D_3+ 500 mg/kg diet MOS	12.26	14.76	2.08	70.90	36.29	88.68	72.98
±SEM	2.03	3.47	0.66	3.10	0.17	1.35	3.05
P value	0.957	0.998	0.883	0.815	0.949	0.926	0.906

[1] *Prepared essential oil.* [2] *Commercial essential oil blend.* [3] *mannan oligosaccharide.* [4] *Pooled SEM.*

Table (5): Effects of dietary supplementation with an essential oil blend and mannan oligosaccharide on some biochemical and hematological parameters of Golden Montaza layers at late phase of egg production.

Item Treatment	Biochemical plasma parameter						
	Total protein g/L	Albumin(A) g/L	Globulin(G) g/L	A/G	ALT U/ml	AST U/ml	Calcium mmol/L
Control diet (D_1)	4.83[b]	2.97	1.87[b]	1.72	25.22	58.22	11.49[b]
D_1+ 200 mg/kg diet PEO[1] (D_2)	4.89[b]	2.93	1.96[b]	1.68	25.33	57.56	11.71[b]
D_1+ 100 mg/kg diet CEO[2] (D_3)	4.99[b]	2.79	2.20[ab]	1.39	24.67	57.44	12.22[ab]
D_1+ 500 mg/kg diet MOS[3]	5.82[a]	3.36	2.47[ab]	1.36	24.67	54.78	12.17[ab]
D_2+ 500 mg/kg diet MOS	5.77[a]	3.07	2.70[a]	1.32	24.89	56.89	12.34[ab]
D_3+ 500 mg/kg diet MOS	5.96[a]	3.18	2.78[a]	1.22	25.33	56.22	12.91[a]
±SEM[4]	0.22	0.15	0.21	0.19	0.61	2.06	0.31
P value	0.00	0.151	0.010	0.354	0.927	0.880	0.035
	Hematological parameters						
	Red blood cells count (10^6/mm^3)	White blood cells count (10^3/mm^3)	Hemoglobin g/dl	Hematocrit (HCT)%	Heterophils (H)	Lymphocyte (L)	H/L ratio
Control diet (D_1)	3.38	7.87	10.11	32.33	18.67	71.78	0.260
D_1+ 200 mg/kg diet PEO (D_2)	3.43	8.58	10.26	33.00	18.00	71.89	0.252
D_1+ 100 mg/kg diet CEO (D_3)	3.76	8.87	10.69	34.22	17.67	73.44	0.241
D_1+ 500 mg/kg diet MOS	3.64	9.31	10.34	32.78	17.89	72.00	0.249
D_2+ 500 mg/kg diet MOS	3.47	8.94	10.51	33.56	17.78	73.11	0.244
D_3+ 500 mg/kg diet MOS	3.74	9.69	10.94	33.78	17.44	73.56	0.238
±SEM[4]	0.11	0.61	0.29	0.94	0.54	0.63	0.01
P value	0.080	0.398	0.371	0.739	0.696	0.152	0.505

[1] Prepared essential oil. [2] Commercial essential oil blend. [3] mannan oligosaccharide. [4] Pooled SEM.

[a–b] Means in a column with different superscripts differ significantly (P≤0.05).

From our results, numerically, it can be observed that laying hens fed diet containing EOs or/and MOS had insignificant lower (P>0.05) values of A/G ratio (Table 5). The A/G ratio has been well known as an indicator for the metabolic activities and immune resistance. In birds, the low A/G ratio indicates more disease resistance and immune response (Griminger, 1986). In the same side, Galal *et al*. (2008) reported that the gamma globulin fraction contains most of the immuno-proteins, including IgM, IgA, IgE and IgG. These usually elevate with ongoing antigenic stimulation, usually from infectious agents. A number of studies conducted showed that MOS had the ability to stimulate elevated antibody levels, especially IgG and IgA levels (Shane, 2001 and Shashidhara and Devagowda, 2003).

b-Hematological parameters: Neither EOB nor MOS had any significant effect on hematological parameters (Table 5). Numerically, laying hens fed diet supplemented with 100 mg CEO +500 mg MOS/kg diet had insignificant higher values of WBCs and Hg, (lower, H, L and H/L ratio). While, those fed control diet had insignificant lower values of RBCs, WBCs, Hg, Ht and L (higher, H and H/L ratio). In this respect, Najafi and Taherpour (2014) reported that the significant improvement in L ratio with oil extract supplementation may be due to herbal oils such as ginger oil increase immunoglobulins levels in the blood as well as the ability to destroy microbial cells by leukocytes due to terpinolen. The present results are in contrast with those of Radwan (2003) who attributed that to the high level of iron in thyme leaves (743 ppm) which may affect the transport of oxygen needed for Hg synthesis in blood. This improvement may be due to the antioxidant activity of EO components, thyme oil (Hertrampf, 2001) and cinnamon oil (Friedman *et al.* 2004). Also, Ibrahim *et al.* (2000) reported that RBCs, Hg and the packed cell volume for rabbits fed diets with 0.5% thyme were significant increased.

c-Plasma lipid and antioxidant profile: Impact of EOB and MOS supplementation on plasma lipid and antioxidant profile of Golden Montazah laying hens at late phase of egg production are shown in Table 6. The results indicated, laying hens fed diet supplemented with 100 mg CEO +500 mg MOS/kg diet had significant higher values of HDL, TAOC and GSH-Px activity (lower values of total lipid, triglycerides and LDL). While, those fed control diet had significant higher values of total lipid, triglycerides and LDL (lower values of HDL, TAOC and GSH-Px activity), as shown in Table 6. From these results, it could be concluded that EOB and MOS may have lowering effect on total lipid, total cholesterol and LDL-cholesterol, thus may be due to the effect of EOs compounds present in these herbs on lipid metabolism). This may lead to produce enriched eggs that are healthier for human consumption and useful for those suffering from heart diseases.

In this respect, Finkel and Holbrook (2000) reported that, enhancement of antioxidant defenses through dietary supplementation would seem to provide a more reasonable and practical approach to reduce the level of oxidative stress and there is a wealth of evidence to support the effectiveness of such a strategy. GSH-Px activity assists in intracellular defence mechanisms against oxidative damage by preventing the production of active oxygen species (Ursini and Bindoli, 1987) and maintain low levels of H_2O_2 and others hydroperoxides in the cell to prevent tissues from peroxidation damages (Kim and Mahan, 2003). α-cymene-2,3-diol (Schwarz *et al.,* 1996) and thymol and carvacrol (Aruoma, 1997 and Baratta *et al.,* 1998) which are found in thyme showed strong antioxidant properties. Farag *et al.* (1989) discussed the relationship between the antioxidant property and the chemical composition of the EOs. It was suggested that the high antioxidant activity of thymol is due to the presence of phenolic OH groups that act as hydrogen donors to the peroxy radicals produced during the first step in lipid oxidation, thus retarding the hydroxyperoxide formation.

Youdim and Deans (2000) investigated the effect of thyme oil and its major compound, thymol, as dietary antioxidant supplements on age-related changes in polyunsaturated fatty acids in various organs. The supplements act as effective free radicals scavengers and influence the in vivo antioxidant defense systems such as superoxide dismutase, GSH-Px and vitamin E.

In accordance with the results of the present experiment, Radwan *et al* (2008), found that supplementation of thyme at 1.0% significantly decreased total lipid, in comparison to the control, while total cholesterol and LDL-cholesterol decreased insignificantly compared to control group.

Table (6): Effects of dietary supplementation with an essential oil blend and mannan oligosaccharide on plasma lipid and antioxidant profile, Toe-web swelling and antibody titer against avian Newcastle and Influenza diseases of Golden Montaza layers at late phase of egg production.

Item	Plasma lipid profile					Plasma antioxidant profile	
Treatment	Total liped mg/dl	Triglycerides mg/dl	Cholesterol Total, mg/dl	Cholesterol Low density lipoprotein	Cholesterol High density lipoprotein	Total antioxidant capacity (m M/l)	Glutathione peroxidase activity mU/ml)
Control diet (D_1)	840.34^{a}	101.47^{a}	185.79	104.06^{a}	55.63^{b}	0.77^{c}	0.68^{c}
D_1+ 200 mg/kg diet PEO[1] (D_2)	795.21^{ab}	99.02^{a}	185.20	95.38^{ab}	59.78^{ab}	0.80^{bc}	0.86^{bc}
D_1+ 100 mg/kg diet CEO[2] (D_3)	738.90^{bc}	91.18^{a}	179.34	77.37^{bc}	60.84^{a}	0.88^{ab}	1.34^{a}
D_1+ 500 mg/kg diet MOS[3]	789.07^{abc}	96.08^{a}	182.96	78.89^{bc}	59.73^{ab}	0.82^{bc}	0.91^{bc}
D_2+ 500 mg/kg diet MOS	718.21^{bc}	88.24^{a}	177.19	75.27^{bc}	59.65^{ab}	0.87^{ab}	1.15^{ab}
D_3+ 500 mg/kg diet MOS	700.36^{c}	71.90^{b}	176.71	64.17^{c}	63.11^{a}	0.95^{a}	1.41^{a}
±SEM[4]	29.63	4.61	6.43	6.54	1.52	0.03	0.13
P value	0.013	0.001	0.855	0.001	0.041	0.00	0.001

	Toe-web swelling (difference)mm (hour)			Antibody titer against avian Newcastle and Influenza diseases	
	24	48	72	Newcastle	influenza
Control diet (D_1)	0.30^{e}	0.22^{d}	0.09^{d}	8.67	6.89^{c}
D_1+ 200 mg/kg diet PEO (D_2)	0.41^{d}	0.35^{c}	0.18^{cd}	8.78	7.11^{bc}
D_1+ 100 mg/kg diet CEO (D_3)	0.45^{cd}	0.38^{bc}	0.23^{bc}	8.89	7.89^{abc}
D_1+ 500 mg/kg diet MOS	0.55^{bc}	0.42^{abc}	0.29^{ab}	9.00	8.11^{ab}
D_2+ 500 mg/kg diet MOS	0.63^{ab}	0.48^{ab}	0.32^{ab}	9.00	8.11^{ab}
D_3+ 500 mg/kg diet MOS	0.68^{a}	0.52^{a}	0.36^{a}	9.11	8.33^{a}
±SEM	0.035	0.036	0.032	0.27	0.35
P value	0.00	0.00	0.00	0.867	0.022

[1] *Prepared essential oil.* [2]*Commercial essential oil blend.* [3] *mannan oligosaccharide.* [4]*Pooled SEM.*
[a–d] *Means in a column with different superscripts differ significantly (P≤0.05).*

These results agree to a large extent with those obtained by Ali *et al*. (2007) who found that addition of thyme to hen's diets significantly decreased plasma HDL, total cholesterol, triglyceride and total lipid.

Impact of EOB and MOS supplementation on immune response to antibody titer of Newcastle disease virus (NDV) and Avian Influenza virus (AIV) of Golden Montazah laying hens at late phase of egg production are shown in Table 6. The results indicated, laying hens fed diet supplemented with 100 mg CEO +500 mg MOS/kg diet had significant ($P \leq 0.05$) higher value of immune response to antibody titer of AIV. While, those fed control diet had significant lower value of immune response to antibody titer of AIV, as shown in Table 6. Data revealed that there were no significant differences of NDV among treatment groups. Also, data of NDV indicated that the worst NDV value was observed for control group, while the best NDV was observed for hens fed diet containing 100 mg CEO +500 mg MOS/kg diet. From these data it can be observed that using of MOS alone or in mixture with EOs in diets resulted in an improvement in immune responses to antibody titer of AIV and NDV as compared to those fed control diet.

In this respect, Moran (2004) reported that MOS assisted with antigen processing in order to initiate the early stages of the immune response, MOS served as an immune modulator in laying hens (Cotter *et al.,* 2000) and in broiler breeders (Shashidhara and Devegowda, 2003). The anti-oxidation properties of some herbs bioactive (extracts and EOs) have been thought to have a role in the development of immune response in birds via protecting cells from oxidative damage and enhancing the function and proliferation of these cells (Ma *et al.,* 2005). However, Ozek *et al.* (2011) demonstrated that an EO blend was not effective in improving the humoral immune response of layer hens as measured serum IBDV, NDV and IBV titers. Also, Bozkurt *et al.* (2012) found that none of MOS or EOs, whether yeast-based or botanical originated, could support the immune system and boost antibody titers.

Effect of experimental treatments on cell-mediated immunity of Golden Montazah laying hens are shown in Table 6. Significantly ($P \leq 0.01$) higher skin thickness was observed at 24, 48 and 72 h post challenge with PHA-P for hens fed diet containing 100 mg CEO +500 mg MOS/kg diet, while, significantly ($P \leq 0.01$) lower one was recorded with control group at 24, 48 and 72 h post challenge. Obviously, hens fed diet added MOS alone or in mixture with EOs had significantly hyper responder to PHA-P injection as compared to those fed control diet.

The skin response reflects a complex series of physiological events such as mitogen-receptor and lymphocyte-macrophage interactions, release of chemical mediators, cellular proliferation and changes in vascularity (Chandra and Newberne, 1977). Histologically, PHA is strongly mitogenic to T-Iymphocytesa and intradermali injections elicit macrophage infiltration and dense perivascular accumulations of lymphocytes 24 h post-injection in chickens (McCorkle *et al.,* 1980). The increased infiltration by basophils and eosinophils 24 h post-injection has been described as a cutaneous basophil hypersensitivity response (Stadeckerm *et al.,* 1977).

In conclusion, the results of this study indicated that feeding Golden Montazah laying hens during the period from 56 to 72 weeks of age on diets containing 100 mg CEO +500 mg MOS/kg diet improved the reproductive performance and increased stimulation of immune, HDL cholesterol, GSH-Px activity, total antioxidants activity in plasma and decreased cholesterol concentration in the egg yolk. This may lead to produce enriched eggs that are healthier for human consumption especially for those suffering from heart diseases.

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تأثير إضافة مزيج من الزيوت الضرورية والمنان أوليجوسكرايد على: ٢- الأداء التناسلي، جودة البيض وبعض قياسات الدم لدجاج المنتزه الذهبي البياض في مرحلة متأخرة من إنتاج البيض

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تم إجراء التجربة في مشروع الدواجن التكاملي محافظة الفيوم – مصر، وذلك خلال الفترة من شهر مارس إلى يوليو لسنة ٢٠١٤.وذلك لدراسة تأثير إضافة مزيج من الزيوت الضرورية والمنان أوليجوسكرايد وخليطيهما على الأداء التناسلي، جودة البيض وبعض صفات الدم لدجاج المنتزه الذهبي البياض في مرحلة متأخرة من إنتاج البيض. تم توزيع ٧٢٠ دجاجة بياضة و ٧٢ ديك من سلالة المنتزه الذهبي عمر ٥٦ أسبوع بصورة عشوائية إلى ست مجموعات تجريبية متساوية (١٢٠ دجاجة + ١٢ ديك) كل معاملة مقسمة إلي ثلاثة مكررات (٤٠ دجاجة + ٤ ديك /مكرر) وتم توزيع المكررات علي ١٨ حظيرة أمهات وكانت الدجاجات متماثلة في وزن الجسم وإنتاج البيض. وكانت المعاملات التجريبية كالتالي :

١- غذيت الطيور علي عليقة المقارنة. ٢-عليقة ١+ ٢٠٠ مللجم مزيج الزيوت الضرورية المحضرة /كجم عليقة.

٣-عليقة ١+ ١٠٠ مللجم مزيج الزيوت الضرورية التجارية /كجم عليقة. ٤-عليقة ١+ ٥٠٠ مللجم منان اوليجوسكريد /كجم عليقة.

٥-عليقة ١+ ٢٠٠ مللجم مزيج الزيوت الضرورية المحضرة + ٥٠٠ مللجم منان اوليجوسكريد /كجم عليقة.

٦-عليقة ١+ ١٠٠ مللجم مزيج الزيوت الضرورية التجارية + ٥٠٠ مللجم منان اوليجوسكريد /كجم عليقة.

وتم تلخيص النتائج المتحصل عليها كما يلي :

لم يكن هناك أي فرق معنوي لجميع المعاملات التجريبية بالنسبة لجودة البيض، عند ٦٤ و٧٢ أسبوع، نسبة الاخصاب والفقس% عند ٦٤، ٦٨ و ٧٢ أسبوع. لم يكن هناك أي فرق معنوي لجميع المعاملات التجريبية بالنسبة للتركيب الكيمائي للبيض فيما عدا تركيز الكوليستيرول في الصفار عند عمر ٧٢ أسبوع، فكانت الدجاجات المغذاة علي ١٠٠ مللجم مزيج الزيوت الضرورية التجارية + ٥٠٠ مللجم منان اوليجوسكريد /كجم عليقة أقل نسبة كوليستيرول للصفار، بينما كانت للدجاجات المغذاة علي عليقة المقارنة اعلي نسبة كوليستيرول عند عمر ٧٢ أسبوع.

لم تكن هناك أي فروق معنوية نتيجة لإضافة مزيج من الزيوت الضرورية والمنان أوليجوسكريد علي القياسات البيوكيمائية لبلازما الدم فيما عدا البروتين الكلي، الجلوبيولين والكالسيوم والتي تأثرت معنوياً. فكانت الدجاجات المغذاة علي ١٠٠ مللجم مزيج الزيوت الضرورية التجارية + ٥٠٠ مللجم منان اوليجوسكريد /كجم عليقة أعلي قيم، بينما كان للدجاجات المغذاة علي عليقة المقارنة اقل قيم لهذه القياسات. لم تكن هناك أي فروق معنوية نتيجة لإضافة مزيج من الزيوت الضرورية والمنان أوليجوسكريد علي Hematological parameters كانت الدجاجات المغذاة علي ١٠٠ مللجم مزيج الزيوت الضرورية التجارية + ٥٠٠ مللجم منان اوليجوسكريد /كجم عليقة أعلي نسبة للكوليستيرول عالي الكثافة، كثافة مضادات الأكسدة الكلية، الجلسريدات الثلاثية ونشاط إنزيم الجلوتاثيون في بلازما الدم. بينما كان للدجاجات المغذاة علي عليقة المقارنة اعلي قيم للدهون الكلية، الجلسريدات الثلاثية و الكوليستيرول منخفض الكثافة في بلازما الدم.

كانت الدجاجات المغذاة علي ١٠٠ مللجم مزيج الزيوت الضرورية التجارية + ٥٠٠ مللجم منان اوليجوسكريد /كجم عليقة الأعلي معنوياً بالنسبة للاستجابة المعنوية لمستوي الأجسام المناعية لفيروس أنفلونزا الطيور. بينما كانت للدجاجات المغذاة علي عليقة المقارنة الأقل معنوياً بالنسبة للاستجابة المعنوية لمستوي الأجسام المناعية لفيروس أنفلونزا الطيور. لم تكن هناك أي فروق معنوية بين المعاملات التجريبية بالنسبة لفيروس النيوكسل.

يمكن التوصية بأن تغذية الدجاج البياض خلال الفترة من ٥٦ إلي ٧٢ أسبوع من العمر من سلالة المنتزه الذهبي علي عليقة تحوي ١٠٠ مللجم مزيج الزيوت الأساسية التجارية + ٥٠٠ مللجم منان اوليجوسكريد /كجم عليقة حسنت الأداء التناسلي للدجاج البياض وزادت الجسام المناعة، الكوليستيرول عالي الكثافة، مضادات الأكسدة الكلية ونشاط إنزيم الجلوتاثيون في بلازما الدم،خفض كوليستيرول صفار البيض وهذا يودي إلي إنتاج بيض صحي للاستهلاك الأدمي وخاصة مرضي القلب.