

EFFECT OF DIETARY SUPPLEMENTATION WITH AN ESSENTIAL OILS BLEND AND MANNAN OLIGOSACCHARIDE ON: 1- THE PRODUCTIVE PERFORMANCE OF GOLDEN MONTAZA LAYERS AT LATE FACE OF EGG PRODUCTION

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(Received 16/6/2015, accepted 30/7/2015)

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 productive performance of Golden Montazah laying hens at late egg production period. A total number of 720 laying hens plus 72 males of Golden Montaza at 56 weeks of age were allocated randomly into six treatments groups (120 hens plus 12 males), each group was equally subdivided into three replicates (40 hens plus four males). Birds were distributed into 18 pens (40 hens and four males 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: Laying hens fed diet containing 100 mg CEO + 500 mg MOS/kg diet had significantly higher values of egg production (EP%), live body weight change (LBWC) and the best feed conversion (FC), crude protein conversion (CPC) and caloric conversion ratio (CCR) during all significant periods studied, while, laying hens fed control diet had significantly lower values of EP%, LBWC and the worst FC, CPC and CCR during the same periods. No significant effect were observed for egg mass, feed intake, initial body weight, final body weight and mortality rate% during the experimental period. There were insignificant differences among all dietary treatments in carcass characteristics except, spleen%. Laying hens fed diet containing 100 mg CEO +500 mg MOS/kg diet had higher spleen% whereas, those fed control diet had lower spleen% at the end of experimental period. Laying hens fed diet containing 100 mg CEO + 500 mg MOS/kg diet had significantly higher values of lactic acid bacteria count and the lower values of total count of bacteria and *E-coli*, while, those fed control diet had significantly higher values of total count of bacteria and *E-coli* and the lower values of lactic acid bacteria count. Golden Montazah laying hens fed diet containing 100 mg CEO +500 mg MOS/kg diet gave the best economical and relative efficiency values during the period from 56 to 72 weeks. In conclusion, the results of this study indicated that feeding Golden Montazah laying hens on diets containing 100 mg CEO +500 mg MOS/kg diet improved the productive performance and relative economic efficiency values of laying hen during the period from 56 to 72 weeks of age.

Key words: *Essential oil blend, mannan oligosaccharides, laying hens and egg production.*

INTRODUCTION

In poultry production since banning antibiotic growth promoters in poultry feeds, there are a number of non-therapeutic alternatives to antibiotic growth stimulators, including enzymes, probiotics, prebiotics, immune stimulants, acidifiers, antioxidants and phytogetic additives (Peric *et al.*, 2009 and Awadein *et al.*, 2010).

Phytogetic additives, as natural substances, have been recognized as a promising alternative solution, as they meet the requirements of consumers in terms of food safety and solve the problem of bacteria

resistance by pathogenic bacteria that occurs as a result of using antibiotics as growth promoters (Silva Cardoso *et al.*, 2012). Miller *et al.* (1993) indicated that farm animals in intensive farming systems are frequently exposed to stress. Deficiencies of natural protective substances or excess exposure to stimulators of reactive oxygen metabolites production, may increase oxidative damage to important biological macromolecules such as proteins, DNA, lipids and affecting their normal function and consequently leading to reduce performance or disease (Valko *et al.*, 2007 and Gaedicke *et al.*, 2009). Apart from polyunsaturated fatty acids, other factors such as nutrition, environmental temperature and ethological stress can cause oxidative stress and increase the requirement for antioxidant supplementation. Over the past decade, extracts and essential oils (EOs) of some herbs have received growing attention as a possible means of stimulating growth in farm animals. Promising results were reported on performance of laying hens fed diets supplemented with EOs (Cabuk *et al.*, 2006; Bolukbasi *et al.*, 2007; 2008 and Bozkurt *et al.*, 2012 a).

Mannan oligosaccharides (MOS) are complex sugars and are extracted from the cell wall of either *Saccharomyces cerevisiae* or *Saccharomyces boulardii* yeasts (Hofacre *et al.*, 2003). Several comprehensive reviews (Berry and Lui, 2000; Stanley *et al.*, 2000; Shashidhara and Devegowda, 2003 and Gurbuz *et al.*, 2011) on the use of commercially available MOS in layer and broiler breeder diets reported that MOS can improve egg production performance and immunity. Kocher *et al.* (2004) acknowledged that MOS can influence the utilization of nutrients in the intestines, and was capable of stimulating specific microbial populations resulting in improved fiber fermentation with a reduction in starch and sugar utilizing bacterial populations.

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 productive performance 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 productive performance of Golden Montazah laying hens at the late egg production period. 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 weeks of age, experimental hens were selected from the Golden Montaza stock reared at TPP, Fayoum, Egypt 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 males of Golden Montaza at 56 weeks of age were allocated randomly into six treatments groups (120 hens plus 12 male), each group was equally subdivided into three replicates (40 hens plus four males). Birds were distributed into 18 pens (40 hens and four males each) in such order to have a similar mean BW and average daily EP.

All birds were reared under the same management conditions in similar open-sided floor pens with (3×2.5 m; 6 birds/m²). 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.

The birds were reared under the normal environmental conditions of TPP farms. 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 hours of light per day (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 of age.

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 (Enviva EO 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 iso-caloric) of Golden Montazah laying hens (16% CP and 2750 Kcal ME/Kg diet) according to the 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. The experimental diets were weighed daily and their residues left in troughs were weighed at the end of each 7 days interval for each pen and the consumed feed was calculated.

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.

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 ₂ SO ₄	0.05
Total	100
<u>Calculated analyses% (according to NRC, 1994):</u>	
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. D₃, 3300000 I.U.; Vit. E, 60000 mg; Vit. K₃, 3600 mg; Vit. B₁, 2200 mg; Vit. B₂, 12000 mg; Vit. B₆, 5500 mg; Vit. B₁₂, 20 mg; biotin, 250mg; folic acid, 1500 mg; pantothenic acid, 15000 mg; Zn, 80000 mg; Mn, 100000 mg; Fe, 80000 mg; Cu, 9000 mg; I, 1100 mg; Co, 200mg; Se, 300mg; 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).

Individual BW were recorded at the beginning and the end of the experiment to the nearest g in the early morning before receiving any feed and water to calculate live body weight changes (LBWC). Egg number (EN) and egg weight (EW) were recorded daily to calculate egg production% (EP%, as hen house) and egg mass (EM= EN* EW). Feed intake (FI) was recorded weekly and it using to calculate feed conversion (FC=FI/EM), crude protein conversion (CPC= FI*CP%/EM) and caloric conversion ratio

(CCR= FI*ME. K cal/EM). Mortality% was recorded daily and calculated for each treatment of the experimental birds during the whole experimental period by subtracting the final number of live birds at the end of a certain period from the initial number of live birds at the beginning of the same period.

At the end of the experimental period, a slaughter test was performed on 18 hens (three hens from each treatment) whose body weight was closest to the replicate mean, selected birds were deprived of feed for 8 hours, then individually weighted and slaughtered by a sharp knife and allowed to bleed freely (customary Islamic way), then feathers were plucked via Chicken Feather Removal Machine after scalding. Carcasses were manually eviscerated (head, viscera and shanks were removed).

Carcasses were left for one hour to remove excess water then weighted. Dressing% was calculated without giblets (heart, gizzard, liver and spleen) and abdominal fat, then the weight of each part was calculated as percentage of the LBW.

At the time of slaughter, intestine was removed and the digest contents of this intestinal segment (1 g) were collected and homogenized with 10 ml phosphate buffer solution. The digest specimens were sent packed on ice to the laboratory (Microbiological Laboratory, Reference laboratory for veterinary Quality Control on poultry production, Animal Health Research Institute) for enumeration of total bacteria, *E. coli* and *Lactobacilli spp.* All the data are expressed as CFU/g $\times 10^4$ (colony forming units), i.e. units that form colonies (Barnes and Impey, 1970).

Economical efficiency of EP was calculated from the input-output analysis using data from feeding expenses, from eggs selling incomes, finally achieving the absolute revenue. Other costs (biological material, husbandry expenses, medical treatments, wages etc.), have not been considered, knowing they were identical for both groups during the entire experimental period. These values were calculated as the net revenue per unit of total costs (LE/hen) depending on the local market prices of the ingredients used for formulating the experimental diet (the average basal diet cost was 2798 LE/ton). Prices of supplementations cinnamon oil, thyme oil, commercial oil and MOS were 360, 320, 280 and 80 LE/Kg, respectively. Also, the total income (LE/hen) was calculated depending on the average market price of both hatching and table eggs (1.20 and 0.68 L.E, respectively). The percentage of hatching and table eggs from the total eggs produced assumed as 88% and 12%, respectively. It has been calculated in accordance with the Technical Efficiency Regulations for the TPP.

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) and e_{ij} : random error. Treatment means indicating significant differences ($P \leq 0.01$ and $P \leq 0.05$) were tested using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Laying hens productive performance:

Effects of dietary supplementation with an EOB and MOS on EP%, EM, FI and FC of Golden Montazah laying hens at late phase of egg production are shown in Tables 2 and 3. Dietary EOB and MOS effect were significant ($P \leq 0.01$) for EP% and FC during all periods studied except, the period from 56 to 60 weeks of age (Tables 2 and 3). It is clear that, laying hens fed diet containing 100 mg CEO + 500 mg MOS/kg diet had significantly higher values of EP% and the best FC during all significant periods studied, while, laying hens fed control diet had significantly lower values of EP% and the worst FC during the same periods (Tables 2 and 3). Numerically, laying hens fed diet containing 100 mg CEO + 500 mg MOS/kg diet had higher values of EP% and the best FC during the period 56 to 60 weeks of age, while, laying hens fed control diet had lower values of EP% and the worst FC during the same period (the difference is not significant). Presumably the increased EP% and improved FC as a result of adding MOS and may improve digestive enzyme activity, gastrointestinal microflora and intestinal morphology, and thereby enhance digestion and absorption of nutrients (Spring *et al.*, 2000 and Yang *et al.*, 2008).

Similar results of significant FC improvement were found in Japanese quail and laying hens were

reported by Ghosh *et al.* (2007) and Hassan and Ragab (2007). Also, Berry and Lui (2000) and Stanley *et al.* (2000) reported considerable improvement in EP% in the MOS-fed birds. This is may be due to MOS maintain gut health by adsorption of pathogenic bacteria containing type-1 fimbriae of different bacterial strains and remove the bacteria from gut (Oyofa *et al.*, 1989 and Spring *et al.*, 2000) and increase villus height, uniformity and integrity also, increase in crypt depth is attributed to greater expenditure of energy to develop the absorptive surface (Oliveira *et al.*, 2006; Mourao *et al.*, 2006 and Ghosh *et al.*, 2007).

On the other hand, Bio-Mos supports β -glucans in stimulating defence mechanisms in the body, activating digestive enzymes, increasing the absorption of nutrients from feed owing to its ability to bind pathogenic bacteria and neutralizing toxins excreted by such bacteria (Oguz and Parlat, 2004 and Zaghini *et al.*, 2005). This lead to improved poultry performance and lowered susceptibility to infections of the digestive and respiratory systems (Mikulski *et al.*, 2008).

Table (2): Effects of dietary supplementation with an essential oil blend and mannan oligosaccharide on egg production%, and egg mass of Golden Montazah laying hens at late phase of egg production.

Treatments	Age period, weeks				
	56-60	61-64	65-68	69-72	56-72
	Egg production%				
Control diet (D ₁)	50.09	44.76 ^c	39.35 ^b	33.81 ^d	42.00 ^d
D ₁ + 200 mg PEO ¹ /kg diet (D ₂)	50.74	45.51 ^{bc}	41.82 ^b	35.48 ^d	43.39 ^{cd}
D ₁ + 100 mg CEO ² /kg diet (D ₃)	51.55	49.11 ^{ab}	47.20 ^a	42.08 ^{bc}	47.49 ^b
D ₁ + 500 mg MOS ³ /kg diet	50.89	45.95 ^{bc}	43.18 ^b	40.06 ^c	45.02 ^c
D ₂ + 500 mg MOS /kg diet	51.93	49.85 ^a	48.04 ^a	44.26 ^{ab}	48.52 ^{ab}
D ₃ + 500 mg MOS /kg diet	52.29	51.19 ^a	49.26 ^a	46.34 ^a	49.77 ^a
±SEM ⁴	0.63	1.14	1.27	0.78	0.55
P value	0.21	0.008	0.001	0.000	0.000
	Egg mass (g/hen/day)				
Control diet (D ₁)	54.81	54.25	53.86	53.14	54.02
D ₁ + 200 mg PEO/kg diet (D ₂)	55.27	54.50	54.24	53.56	54.39
D ₁ + 100 mg CEO/kg diet (D ₃)	54.94	54.98	54.23	53.73	54.47
D ₁ + 500 mg MOS/kg diet	55.09	55.25	54.93	54.23	54.87
D ₂ + 500 mg MOS /kg diet	55.22	55.62	54.43	54.96	55.06
D ₃ + 500 mg MOS /kg diet	56.00	55.62	55.44	54.82	55.47
±SEM	0.50	0.55	0.64	0.73	0.53
P value	0.626	0.419	0.572	0.471	0.480

¹Prepared essential oil ² Commercial essential oil blend ³ mannan oligosaccharide

⁴Pooled SEM

^{a-d} Means in a column with different superscripts differ significantly ($P \leq 0.05$).

Furthermore, Hertrampf (2001) and Williams and Losa (2001) reported that an important claim often made of phytogetic feed additives is improving efficiency of FC thereby enhancing the intestinal availability of essential nutrients for absorption however, the specific experimental verification in laying hens is rather limited. Although the considerable potential to support gut health (Sims *et al.*, 2004 and Baurhoo *et al.*, 2009). The results of the present study did not confirm the findings of Shashidhara and Devegowda (2003) and Hassan and Ragab (2007) who found that MOS supplementation had no influence on EP% of laying hens.

In the present study, either EOB or MOS and their mixture were effective in promoting EP% and FC of Golden Montazah layer. In accordance with the results of the present experiment, similar improvements on laying performance of brown (Cabuk *et al.*, 2006) and white (Bolukbasi *et al.*, 2007) layers were observed in previous works. Specifically, the dietary supplementation of mixed EOs and thyme oil improved EP% by 5.43% and 6.20%, respectively. Similar observation was reported by Ather (2000), who determined that the addition of an EOs combination to the broiler breeder diet resulted in remarkable improvements on EP%.

Ali *et al.* (2007) found that numerically increased egg number and improved FC compared to hens fed basal diet. Moreno *et al.* (2006), reported that carnosic acid and rosmarinic acid may be the main bioactive antimicrobial compounds present in rosemary. Dietary feeding of EOs extracted from herbs

improved the secretion of digestive enzymes, so improved the digestibility of the feeds and improved the performance for broiler (Hernandez *et al.*, 2004 and Jang *et al.*, 2004).

No significant effect were observed for EM and FI during the experimental period (Table 2). Numerically, results in Table 2 showed that laying hens fed diet containing 100 mg/kg diet CEO +500 mg/kg diet MOS had higher EM values during the experimental periods studied, except, the period from 69 to 72 week of age (laying hens fed diet containing 200 mg/kg diet PEO +500 mg/kg diet MOS had higher EM). While, those fed control diet had lower values of EM during all periods studied (the difference is not significant). Similarly, MOS supplementation had no significant effects on FI (Hassan and Ragab, 2007).

In this respect, Williams and Losa (2001) and Windisch *et al.* (2008) reported that the assumption that extract and EO of some herbs might improve the palatability of feed due to their aromatic characteristics could promote feed consumption when added to diets of poultry. Nevertheless, such an assumption has not yet been confirmed with consistent and repeatable evidences in laying hens. A significant stimulation of FI was not obtained in any of the studies when laying hens administered with EOs of oregano (Florou-Paneri *et al.*, 2005), thyme, sage, and rosemary (Bolukbasi *et al.*, 2008) and a blend of EOs (Ozek *et al.*, 2011 and Bozkurt *et al.*, 2012 a). Available limited results do not also support the promotion in FI in layer hens with respect to dietary treatment with MOS (Berry and Lui, 2000; Stanley *et al.*, 2000; Zaghini *et al.*, 2005 and Cabuk *et al.*, 2006).

Radwan *et al.* (2008), found that supplementation of herbs improved production performance compared with control group. The beneficial effect of *labiatae* family (thyme, rosemary and oregano) may be due to the phenolic compounds which considerably exhibit antimicrobial and antifungal activity (Giannenas *et al.*, 2003; Arcila-Lozano *et al.*, 2004 and Bozin *et al.*, 2006). This activity may be due to thymol and carvacrol which are present in the EOs of thyme and oregano (Basilico and Basilico, 1999). In addition, Abdel-Latif *et al.* (2002) attributed the improvement in growth and FC of chicks fed thyme leaves to the enhancement in thyroid activity and the biological role of such medicinal plant in the metabolic functions and biosynthesis of hormones.

Table (3): Effects of dietary supplementation with an essential oil blend and mannan oligosaccharide on feed intake and feed conversion ratio of Golden Montazah laying hens at late phase of egg production.

Treatments	Age period, weeks				
	56-60	61-64	65-68	69-72	56-72
	Feed intake (g, hen/day)				
Control diet (D ₁)	119.32	112.02	108.14	105.16	111.16
D ₁ + 200 mg PEO ¹ /kg diet (D ₂)	117.39	113.24	110.81	110.32	112.94
D ₁ + 100 mg CEO ² /kg diet (D ₃)	117.26	111.05	110.02	106.71	111.26
D ₁ + 500 mg MOS ³ /kg diet	113.09	111.23	104.36	111.42	110.03
D ₂ + 500 mg MOS /kg diet	118.47	112.45	107.76	107.97	111.66
D ₃ + 500 mg MOS /kg diet	118.17	113.50	107.52	112.01	112.80
±SEM ⁴	2.51	1.55	1.97	2.14	0.81
P value	0.595	0.821	0.322	0.223	0.179
	Feed conversion ratio (g feed/g egg mass)				
Control diet (D ₁)	4.35	4.61 ^a	5.13 ^a	5.85 ^a	4.89 ^a
D ₁ + 200 mg PEO/kg diet (D ₂)	4.19	4.57 ^a	4.90 ^{ab}	5.83 ^a	4.78 ^a
D ₁ + 100 mg CEO/kg diet (D ₃)	4.15	4.12 ^{bc}	4.31 ^c	4.72 ^{bc}	4.30 ^{bc}
D ₁ + 500 mg MOS/kg diet	4.04	4.39 ^{ab}	4.40 ^{bc}	5.14 ^b	4.45 ^b
D ₂ + 500 mg MOS /kg diet	4.13	4.06 ^c	4.12 ^c	4.44 ^c	4.18 ^c
D ₃ + 500 mg MOS /kg diet	4.04	3.99 ^c	3.94 ^c	4.41 ^c	4.09 ^c
±SEM	0.12	0.10	0.16	0.19	0.08
P value	0.508	0.002	0.002	0.000	0.000

¹Prepared essential oil ² Commercial essential oil blend ³ mannan oligosaccharide

⁴Pooled SE

^{a-c} Means in a column with different superscripts differ significantly ($P \leq 0.05$).

Effects of dietary supplementation with an EOB and MOS on crude protein conversion (CPC) and caloric conversion ratio (CCR) of Golden Montazah laying hens at late phase of EP are shown in Table 4. Results in Table 4 showed that inclusion of PEO, CEO and MOS in laying hens diets caused significant

($P \leq 0.01$) differences in CPC and CCR during all periods studied except, the period from 56 to 60 weeks of age. It is clear that, laying hens fed diet containing 100 mg CEO +500 mg MOS/kg diet had significantly the best values of CPC and CCR during all periods studied (this result may be due to the high EP% values recorded for this group during these periods), while, laying hens fed control diet had significantly the worst values of CPC and CCR during the same periods (Table 4).

The response observed in present study agree with those reported by Samarasinghe *et al.* (2003) and Hassan and Ragab (2007) who reported that, adding MOS to diets of laying hens significantly improved CPC and CCR than the unsupplemented diet.

Results presented in Table (5) showed that there were insignificant differences among all dietary treatments in initial and final body weight and mortality rate%. Inclusion of PEO, CEO and MOS in laying hens diets caused significant ($P \leq 0.01$) differences in live body weight change (LBWC). It is clear that laying hens fed diet containing 100 mg CEO +500 mg MOS/kg diet had lower LBWC whereas, those fed control diet had higher LBWC during the experimental period.

Table (4): Effects of dietary supplementation with an essential oil blend and mannan oligosaccharide on crude protein conversion ratio and caloric conversion ratio of Golden Montazah laying hens at late phase of egg production.

Treatments	Age period, weeks				
	56-60	61-64	65-68	69-72	56-72
	Crude protein conversion ratio				
Control diet (D ₁)	0.70	0.74 ^a	0.82 ^a	0.94 ^a	0.78 ^a
D ₁ + 200 mg PEO ¹ /kg diet (D ₂)	0.67	0.73 ^a	0.78 ^{ab}	0.93 ^a	0.76 ^a
D ₁ + 100 mg CEO ² /kg diet (D ₃)	0.66	0.66 ^{bc}	0.69 ^c	0.76 ^{bc}	0.69 ^{bc}
D ₁ + 500 mg MOS ³ /kg diet	0.65	0.70 ^{ab}	0.70 ^{bc}	0.82 ^b	0.71 ^b
D ₂ + 500 mg MOS /kg diet	0.66	0.65 ^c	0.66 ^c	0.71 ^c	0.67 ^c
D ₃ + 500 mg MOS /kg diet	0.65	0.64 ^c	0.63 ^c	0.71 ^c	0.65 ^c
±SEM ⁴	0.02	0.02	0.03	0.03	0.01
P value	0.508	0.002	0.002	0.000	0.000
	Caloric conversion ratio				
Control diet (D ₁)	11.96	12.69 ^a	14.10 ^a	16.08 ^a	13.45 ^a
D ₁ + 200 mg PEO/kg diet (D ₂)	11.51	12.57 ^a	13.48 ^{ab}	16.04 ^a	13.14 ^a
D ₁ + 100 mg CEO/kg diet (D ₃)	11.40	11.32 ^{bc}	11.84 ^c	12.99 ^{bc}	11.83 ^{bc}
D ₁ + 500 mg MOS/kg diet	11.10	12.08 ^{ab}	12.11 ^{bc}	14.13 ^b	12.24 ^b
D ₂ + 500 mg MOS /kg diet	11.36	11.15 ^c	11.34 ^c	12.22 ^c	11.49 ^c
D ₃ + 500 mg MOS /kg diet	11.10	10.97 ^c	10.83 ^c	12.12 ^c	11.23 ^c
±SEM	0.33	0.28	0.45	0.51	0.21
P value	0.508	0.002	0.002	0.000	0.000

¹Prepared essential oil ² Commercial essential oil blend ³ mannan oligosaccharide ⁴ Pooled SE
^{a-c} Means in a column with different superscripts differ significantly ($P \leq 0.05$).

Confirming these findings, Bozkurt *et al.* (2012b) found that the final BW did not differ among treatments. These results imply that hens fed on MOS and EOM strive to cope with the detrimental effects of heat stress without sacrificing performance while keeping BW over the untreated hens. Previous studies had also reported no beneficial effects on the BW of hens when they were fed diets supplemented with oregano EOs (Florou-Paneri *et al.*, 2005) and MOS (Stanley *et al.*, 2000 and Gurbuz *et al.*, 2011).

In contrast to our result, Cabuk *et al.* (2006) reported that the final BW of layer hens fed EOM or MOS diets was significantly higher than those receiving an unsupplemented diet reared under severe hot climatic conditions. While, Ali *et al.* (2007), found that addition of 0.25% thyme leaves to laying hens, significantly decreased LBWC. Also, the response observed in present study disagree with those reported by Hassan and Ragab (2007) who reported that, the MOS supplementation had no significant effects on LBWC of laying hens.

Mortality rate in this study was within the normal range (Table 5), the low MR% obtained in the present study may be related to the tip-top management and first-rate hygiene practices during the entire experimental period. Results revealed that mortality was found to be not related to dietary treatments. The

majority of the total hen mortalities (3 of the 5) occurred in the 12nd week of the experimental period (the last week of the hottest June), meanwhile week of the experiment.

There were insignificant differences among all dietary treatments in carcass characteristics except, spleen%. It is clear that laying hens fed diet containing 100 mg CEO +500 mg MOS/kg diet had higher spleen% whereas, those fed control diet had lower spleen% at the end of experimental period. Numerically, laying hens fed diet containing 100 mg CEO/kg diet had higher dressing%, followed by those fed diet containing 200 mg PEO +500 mg MOS/kg diet, then laying hens fed diet containing 100 mg CEO +500 mg MOS/kg diet but the differences is not significant (Table 6).

Table (5): Effects of dietary supplementation with an essential oil blend and mannan oligosaccharide on body weight change(kg) and mortality rate% of Golden Montazah laying hens at late phase of egg production.

Treatments	Body weight change(kg)			Mortality rate%
	Initial body weight	Final body weight	Body weight change	
Control diet (D ₁)	2.130	2.376	0.246 ^a	0.80
D ₁ + 200 mg PEO ¹ /kg diet (D ₂)	2.146	2.387	0.241 ^{ab}	0.80
D ₁ + 100 mg CEO ² /kg diet (D ₃)	2.085	2.306	0.221 ^{bc}	0.80
D ₁ + 500 mg MOS ³ /kg diet	2.114	2.337	0.222 ^{bc}	0.00
D ₂ + 500 mg MOS /kg diet	2.142	2.357	0.215 ^{cd}	0.80
D ₃ + 500 mg MOS /kg diet	2.137	2.331	0.195 ^d	0.80
±SEM ⁴	0.017	0.018	0.007	0.007
P value	0.202	0.076	0.002	0.956

¹Prepared essential oil; ²Commercial essential oil blend; ³mannan oligosaccharide; ⁴Pooled SE

^{a-d}Means in a column with different superscripts differ significantly ($P \leq 0.05$).

In this respect, Al-Sultan (2003) reported that, higher spleen weight index was observed in birds received feed contained 1.0% turmeric. Low spleen weight could be interpreted as an indicator of low immune activity, because the spleen is a major lymphoid organ in poultry. The decrease of immune tissue weight produces an effect on immune cell phenotypes, immune cell proliferation, and antibody production (Sadeghi *et al.*, 2013).

Table (6): Effects of dietary supplementation with an essential oil blend and mannan oligosaccharide on carcass characteristics of Golden Montazah laying hens at late phase of egg production.

Treatments	Live body weight (g)	Dressed %	Liver %	Heart %	Gizzard %	Abdominal fat%	Spleen%
Control diet (D ₁)	2083	64.73	1.93	0.43	2.01	5.34	0.103 ^c
D ₁ + 200 mg PEO ¹ /kg diet (D ₂)	2100	62.59	2.53	0.43	1.94	5.35	0.117 ^c
D ₁ + 100 mg CEO ² /kg diet (D ₃)	2050	66.14	1.72	0.51	2.10	5.26	0.136 ^{bc}
D ₁ + 500 mg MOS ³ /kg diet	2083	63.07	1.87	0.49	1.93	5.39	0.163 ^{ab}
D ₂ + 500 mg MOS /kg diet	2067	65.74	2.86	0.41	2.29	5.84	0.134 ^{bc}
D ₃ + 500 mg MOS /kg diet	2033	65.54	1.96	0.55	2.11	5.44	0.187 ^a
±SEM ⁴	37.88	1.97	0.40	0.07	0.17	0.56	0.013
P value	0.83	0.73	0.35	0.74	0.71	0.98	0.006

¹Prepared essential oil; ²Commercial essential oil blend; ³mannan oligosaccharide; ⁴Pooled SE

^{a-d}Means in a column with different superscripts differ significantly ($P \leq 0.05$).

However, Bozkurt *et al.* (2008) and Sarica *et al.* (2009) reported no significant improvement in carcass weight and dressing% or carcass part percentages due to supplementing MOS. On the other hand, Ali *et al.* (2007) reported that hens fed thyme or anise had no significant effect on carcass parameters and internal organ. While, the present results disagree with those of Bonos *et al.* (2010), who found that the

addition of MOS significantly ($P \leq 0.01$) increased carcass weight and decreased liver%, but had no effect ($P > 0.05$) on the other parameters (dressing, breast and heart%). Mohamed *et al.* (2008) reported that the highest abdominal fat% was recorded for birds fed the control diet while the lowest value was recorded for birds fed the MOS supplemented diet. Toghyani *et al.* (2011) indicated that no clear mechanism has been reported responsible for the reduction of lipid synthesis by prebiotics and herb oligosaccharides, it might be due to increasing beneficial bacteria such as *Lactobacillus* that decrease the activity of acetyl-Co A carboxylase, which is the rate-limiting enzyme in fatty acids synthesis

Effects of dietary supplementation with an EOB and MOS on intestinal bacterial of Golden Montazah laying hens at late phase of EP are shown in Table 7. It is clear that, laying hens fed diet containing 100 mg CEO + 500 mg MOS/kg diet had significantly ($P \leq 0.01$ and $P \leq 0.05$) higher values of lactic acid bacteria count and the lower values of total count of bacteria and *E-coli*, while, those fed control diet had significantly ($P \leq 0.01$ and $P \leq 0.05$) higher values of total count of bacteria and *E-coli* and the lower values of lactic acid bacteria count at the end of experiment (Table 7). So, results indicated that adding EOB and MOS to laying hens diets each alone or mixture reducing *E. coli* content of the gastrointestinal tract.

In broilers fed a diet containing MOS, a significant drop in the number of pathogenic strains of *Sal.* and *E. coli* was observed, as well as an increase in the desirable intestinal bacteria of *Lactobacillus* spp. and *Bifidobacterium* types, which produce short-chain fatty acids and reduce the pH of the intestinal contents and thus create an unfavourable environment for pathogens (Baurhoo *et al.*, 2007). Hence, improved rearing effects are related to the beneficial influence of the components of the cellular wall of *Saccharomyces cerevisiae* yeast on the development, morphology and metabolism indices in turkeys and chickens (Zaghini *et al.*, 2005). Changes in the morphology of intestines and metabolic indices resulted in better digestion, better absorption of nutrients in the small intestine, improved specific immunity and increased weight gains (Lecewicz *et al.*, 2008).

Table (7): Effects of dietary supplementation with an essential oil blend and mannan oligosaccharide on intestinal bacterial count of Golden Montazah laying hens at late phase of egg production.

Treatment	Total count CFU/g*10 ⁴	E.Coli CFU/g*10 ⁴	lactic acid bacteria CFU/g*10 ⁴
Control diet (D ₁)	9.67 ^a	6.64 ^a	3.70 ^d
D ₁ + 200 mg PEO ¹ /kg diet (D ₂)	9.37 ^a	6.57 ^a	3.97 ^d
D ₁ + 100 mg CEO ² /kg diet (D ₃)	8.60 ^a	5.93 ^{ab}	4.60 ^c
D ₁ + 500 mg MOS ³ /kg diet	7.10 ^b	5.83 ^{ab}	5.13 ^{bc}
D ₂ + 500 mg MOS /kg diet	6.97 ^b	5.69 ^b	5.67 ^b
D ₃ + 500 mg MOS /kg diet	6.37 ^b	5.66 ^b	6.33 ^a
±SEM ⁴	0.47	0.24	0.19
P value	0.001	0.043	0.000

¹Prepared essential oil; ²Commercial essential oil blend; ³mannan oligosaccharide; ⁴Pooled SE

^{a-d}Means in a column with different superscripts differ significantly ($P \leq 0.05$).

In this respect, these results are in good agreement with that of (Al-Kassie, 2010) who studied the effects of the addition of different percent levels of thyme and cinnamon on the intestinal microbial balance on broiler chicks, that include different region of gastro intestinal tract of digestive system (crop, jejunum and large intestine) at six-weeks old of experiment, he found that the two additives (0.5%, 1% thyme and 1% cinnamon) treatments a significant decrease at ($P \leq 0.05$) of total bacteria count (CFU/gm) for in crop, jejunum and large intestine. Also, these results are in good agreement with that of (Bolukbasi and Erhan, 2007 and Al-Kassie, 2008). Where they pointed out the positive effect of thyme additive in decreasing *E. coli* in various of gastrointestinal tract in order to improve the animal health.

The interpretation of these data results reflex the complexity of micro flora development with the age in broiler synchronized with the addition of thyme and its active ingredients (thyme and cervical), inhibition of pathogenic bacteria such as *E. coli* 0157:H7, *Sal. typhimurium*, *Shigella Sonnei* and *Bacillus Subtilis* (Fan and Chen, 2001). *Coli* form bacteria is an indicator tool for intestinal performance, so that thyme and cinnamon on with complex mechanisms affect pathogenic bacteria by changing cell wall bacterial permeability leading to pore formation and osmotic shock and leakage of cytoplasm and its active contents out-side the cell leading to death of them (Lee *et al.*, 2004), the antimicrobial effect

thymol on these bacterin played on vital membrane ions of potassium and hydrogen equilibrium pumps (Bolukbasi and Erhan, 2007).

The same trend was reported with Ali *et al.* (2008), who reported that gram positive bacteria are more sensitive to herbs compounds. Also, Spais *et al.* (2002) found that a commercial feed additive containing herb extracts and organic acids exerts a growth promoting effect comparable to that of *flavomycin*. Moreover, the positive effects of these additives may be explain based on herbal plants and herbal extracts that have appetite effect (Hernandez *et al.*, 2004 and Abou-Sekken *et al.*, 2007) and increased production of digestive enzymes through enhanced liver functions (Williams and Losa, 2001) or antimicrobial activity against pathogenic bacteria which improve the efficiency of feed utilization (Ghazalah and Ibrahim, 1996).

However, Jamroz *et al.* (2003) and Mitsch *et al.* (2004) determined that plant extract (carvacrol, cinnamaldehyde and capsaicin) reduced the total *E. coli* and can control *Clostridium perfringens* colonization in the intestine and feces of broiler chickens. Aantibacterial, anticoccidial, antifungal and antioxidant effects of capsaicin (Chevallier, 1996), thyme oil (Hertrampf, 2001) and cinnamon oil (Friedman *et al.* 2004) were reported. More recently, El-Faham *et al.* (2015) showed that the growth promoting effect of thyme oil on beneficial bacteria (lactic acid) and its growth inhibiting effect on harmful bacteria (*coli*-form) in beneficial in regulating intestinal micro-ecological balance. The beneficial effect of thyme oil can confer protection against potential enter pathogenic bacteria and prevent or cure intestinal diseases.

Economical efficiency (EEf):

Table 8 show the economical efficiency and the relative economical efficiency values. Golden Montazah laying hens fed diet containing 100 mg CEO +500 mg MOS/kg diet gave the best economical and relative efficiency values being 0.751 and 140.13%, respectively followed by hens fed diet containing 100 mg CEO/kg diet being 0.718 and 133.95%, respectively. Hens fed diet 200 mg PEO/kg diet had the worst corresponding values, being 0.525 and 97.90%, respectively. The relative efficiency varied between -2.10 to +40.13% which is of minor importance relative to the other factors of production.

Table (8): Effects of dietary supplementation with an essential oil blend and mannan oligosaccharide on economical efficiency of Golden Montazah laying hens at late phase of egg production.

Items	Treatments					
	Control diet (D ₁)	D1+ 200 mg PEO ¹ /kg diet (D ₂)	D1+ 100 mg CEO ² /kg diet (D ₃)	D1+ 500 mg MOS ³ /kg diet	D ₂ + 500 mg MOS /kg diet	D ₃ + 500 mg MOS /kg diet
Egg production %	42.00	43.39	47.49	45.02	48.52	49.77
TEP ⁴	47.04	48.60	53.19	50.42	54.34	55.74
Total egg Price(L.E.) ⁵	53.51	55.28	60.51	57.36	61.82	63.41
Feed intake (g/hen)	111.16	112.94	111.26	110.03	111.66	112.80
TFI ⁶	12.45	12.65	12.46	12.32	12.51	12.63
Price/ Kg feed (L.E.) ⁷	2.798	2.866	2.826	2.838	2.906	2.866
Total feed cost (L.E.)	34.83	36.25	35.22	34.97	36.34	36.21
Net revenue (L.E.)	18.68	19.03	25.29	22.39	25.48	27.20
Economical efficiency (E.Ef.)	0.536	0.525	0.718	0.640	0.701	0.751
Relative E.Ef.	100.00	97.90	133.95	119.38	130.75	140.13

¹ Prepared essential oil ² Commercial essential oil blend ³ mannan oligosaccharide

⁴ TEP= Total egg production (egg/hen) = EP% X 112 days (Experiment period, days)

⁵ Total egg Price (L.E.) = Table eggs (egg/hen) X0.68 (L.E.) + Hatching eggs (egg/hen) X1.20 (L.E.). Table eggs (egg/hen) = TEP X 12% (assuming12% while soled as Table eggs*), hatching eggs (egg/hen) = TEPX 88% (assuming88% while soled as hatching eggs*). * It has been calculated in accordance with the Technical Efficiency Regulations for the El-Takamoly Poultry project, Fayoum Governorate, Egypt

⁶ TFI = Total feed intake (Kg/hen) = (FI (g/hen/day) /1000) X 112 days (Experiment period, days)

⁷ Price/ Kg feed (L.E.) = Basal diet cost + Supplementation cost

Total feed cost (L.E.) = TFI X Price/ Kg feed (L.E.)

Net revenue (L.E.) = Differences between Total egg Price (L.E.) and Total feed cost (L.E.)

Economical efficiency (E.Ef.) = (Net revenue (L.E.) / Total feed cost (L.E.))

Relative E.Ef.assuming that economical efficiency of the control groups equals 100.

CONCLUSION

The results of this study indicated that feeding Golden Montazah laying hens on diets containing 100 mg CEO +500 mg MOS/kg diet improved the productive, reproductive performance and relative economic efficiency values of laying hen.

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تأثير إضافة مزيج من الزيوت الضرورية والمنان أوليجوسكرايد على: 1- الأداء الإنتاجي 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 2014. وذلك لدراسة تأثير إضافة مزيج من الزيوت الضرورية والمنان أوليجوسكرايد وخليطيهما على الأداء الإنتاجي لدجاج المنتزه الذهبي البياض في مرحلة متأخرة من إنتاج البيض. تم توزيع 720 دجاجة بياضة و 72 ديك من سلالة المنتزه الذهبي عمر 56 أسبوع من العمر بصورة عشوائية إلى ست مجموعات تجريبية متساوية (120 دجاجة + 12 ديك) كل معاملة مقسمة إلى ثلاثة مكررات (40 دجاجة + 4 ديك /مكرر) وتم توزيع المكررات علي 18 حظيرة أمهات وكانت الدجاجات متماثلة في وزن الجسم وإنتاج البيض. وكانت المعاملات التجريبية كالتالي :

- 1- غذيت الطيور علي عليقة المقارنة.
- 2- عليقة 1 + 200 مللجم مزيج الزيوت الضرورية المحضرة /كجم عليقة.
- 3- عليقة 1 + 100 مللجم مزيج الزيوت الضرورية التجارية /كجم عليقة.
- 4- عليقة 1 + 500 مللجم منان اوليجوسكريد /كجم عليقة.
- 5- عليقة 1 + 200 مللجم مزيج الزيوت الضرورية المحضرة + 500 مللجم منان اوليجوسكريد /كجم عليقة.
- 6- عليقة 1 + 100 مللجم مزيج الزيوت الضرورية التجارية + 500 مللجم منان اوليجوسكريد /كجم عليقة.

وتم تلخيص النتائج المتحصل عليها كما يلي :

كانت هناك زيادة معنوية في معدل إنتاج البيض والتغير في وزن الجسم وكذلك أحسن كفاءة تحويل للغذاء، البروتين والطاقة للدجاجات المغذاة علي 100 مللجم مزيج الزيوت الضرورية التجارية + 500 مللجم منان اوليجوسكريد /كجم عليقة خلال الفترات التجريبية، بينما كان للدجاجات المغذاة علي عليقة المقارنة اقل معدل لإنتاج البيض والتغير في وزن الجسم وكذلك أسوء كفاءة تحويل للغذاء، البروتين والطاقة. لم يكن هناك أي تأثير معنوي بالنسبة لكتلة البيض، استهلاك الغذاء، وزن الجسم في بداية التجربة، وزن الجسم في نهاية التجربة ومعدل النفوق خلال الفترة التجريبية.

لم يكن هناك أي فروق معنوية بالنسبة لصفات الذبيحة لكل المعاملات التجريبية فيما عدا النسبة المئوية للطحال فكانت الدجاجات المغذاة علي 100 مللجم مزيج الزيوت الضرورية التجارية + 500 مللجم منان اوليجوسكريد /كجم عليقة أعلى نسبة مئوية للطحال، بينما كانت الدجاجات المغذاة علي عليقة المقارنة اقل نسبة مئوية للطحال في نهاية الفترة التجريبية. الدجاجات المغذاة علي 100 مللجم مزيج الزيوت الضرورية التجارية + 500 مللجم منان اوليجوسكريد /كجم عليقة كانت الأعلى معنويًا في العدد الكلي لبكتريا حامض اللاكتيك والأقل معنويًا في العدد الكلي للبكتريا و *E-coli*. بينما كانت المغذاه علي عليقة المقارنة الأعلى معنويًا في العدد الكلي للبكتريا و *E-coli*. الأقل معنويًا في العدد الكلي للبكتريا حامض اللاكتيك في نهاية الفترة التجريبية. كان للدجاجات المغذاة علي 100 مللجم مزيج الزيوت الضرورية التجارية + 500 مللجم منان اوليجوسكريد /كجم عليقة أحسن كفاءة اقتصادية ونسبية خلال الفترة من 56 إلي 72 أسبوع من العمر.

يمكن التوصية بأن التغذية علي 100 مللجم مزيج الزيوت الضرورية التجارية + 500 مللجم منان اوليجوسكريد /كجم عليقة حسنت الأداء الإنتاجي والكفاءة الاقتصادية والنسبية للدجاج البياض من سلالة المنتزه الذهبي خلال الفترة من 56 إلي 72 أسبوع من العمر.