Supplementation with Probiotic and Peppermint Dry Leaves at Early Laying Period

Ahmed Gouda*(1) Sherin Gabr(2)and Fouad A. Tawfeek(2)

(1). Animal Production Department, Agricultural and Biological Research Institute, National Research Center, Dokki, Cairo 11865, Egypt.
(2). Department of Poultry Breeding Research, Animal Production Research Institute, Ministry of Agriculture, Dokki, Giza 12611, Egypt.
(*Correspondin author: Dr. Ahmed Gouda: E-mail: black_tiger2167@yahoo.com).

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Abstract

This study was conducted to determine the effects of dietary supplementation of probiotic and peppermint dry leaves on productive, physiological and immunological performance of a Mandarah strain during the early laying period. 250 chickens of Mandarah strain, 20 weeks old, were used, and they were divided into five treatments with five replicates in a completely randomized design. The experimental groups were either fed a corn soybean meal basal diet control group (T1) or a basal diet supplemented with either probiotic 0.5g/kg diet (T2), probiotic 1g/kg diet (T3), peppermint 15 g/kg diet (T4), or peppermint 20 g/kg diet (T5). The results obtained confirmed that dietary supplementation of probiotics or peppermint dry leaves to the Mandarah laying hens diets, led to significant improvement ($P<0.0001$) in the measurements of productive, physiological and immunological performance of a Mandarah strain during the early laying period.

Key words: Poultry, dietary supplementations, immunological performance.

Introduction:

The poultry industry is one of the most dynamic of world agribusiness trade, the importance of feed supplements in poultry production has increased in recent years with the aim of improving the economic situation of poultry projects. Nowadays, food safety and the economics of food production are factors that cannot be ignored and taken seriously. Therefore, many countries tend to prohibit antibiotics because of their side effects on both birds and humans, which has encouraged researchers to search for natural means instead. Researchers are looking to find natural materials that are safer than antibiotics as feed additives (Genedy and Zeweil, 2003; Ibrahim *et al*., 2005). Many researchers have indicated the use of probiotics as an alternative to antibiotics this is due to its beneficial effect on improving growth promotion and improvement of feed efficiency in poultry as a result of feeding poultry on microbial culture (El-Sheikh, 2006), microbial balance in the animal's gut, which ensures the presence of beneficial microbes in the gut this would ensure that the animal at all times is healthy, this, of course, cannot be guaranteed under natural conditions. However, if microorganisms and/or substances, which contribute to an appropriate microbial balance, are added to the diet, the animal will continually receive a "boost" to identify the appropriate microbial assemblies, these organisms though will promote metabolism and suppress other undesirable bacteria (El-Sheikh, 2006 and Ali *et al.*, 2017).

The use of medicinal plants in the poultry industry has become popular and requires the selection of the most suitable plant. Various studies have demonstrated the antimicrobial properties of some medicinal plants in the human laboratory. Studies have shown that the use of medicinal plants can play an effective role in producing healthy (organic) products besides improving production. Mint is a member of the Labiate family and is one of the oldest medicinal herbs in the world, this family is characterized by rich in essential oil, has commercial and

medicinal values and widespread throughout the world and are widely use in food, flavor, cosmetic, and pharmaceutical industries (Amanpour *et al*., 2015, Baharvand-Ahmadi *et al.,* 2016 and *Farhadi et al.,* 2016). The chemical components of peppermint are menthol, menthone, cineole, methylacetate, methofuran, isomenthone, limonene, b-pinene, a-pinene, germacrene-d, trans-sabinene hydrate, and pulegone. Menthol is the main phenolic component in oil of peppermint, which has antibacterial activities (Botsoglou *et al.,* 2004, and Cabuk *et al.,* 2006). Studies have shown that this plant has antiseptic, spasmolytic, and disinfectants properties, all of these ingredients have a beneficial effect when used (Nematollah *et al*., 2017).

The present experiment was designed to study the possibility of using probiotic and medicinal plant (peppermint dry leaves) as supplements to Mandarah strain during the early laying period diets to evaluate its effect on productive performance, egg quality, and physiological parameters.

Materials and methods:

Ethical approval

All animal experiments were conducted in accordance with the guidelines and recommendations of the Research Data Preparation Department at the Agricultural Research Center, Egypt and Use Committee and approved by the Animal Ethics Committee of Poultry Breeding, Animal Production Research Institute, Ministry of Agriculture, (approval No. 429/3/11/1).

Animals, diets, and management

The present experiment was conducted by the Department of Animal Production, the National Research Centre, and Department of Poultry Breeding, Animal Production Research Institute, Ministry of Agriculture, The Animal. 250 chickens of the Mandarah strain, 20 weeks old, were used, and they were divided into five treatments with five replicates in a completely randomized design. The experimental groups were either fed a corn soybean meal basal diet control group **(T1)** or a basal diet supplemented with either Probiotic 0.5g / kg diet **(T2)**, Probiotic 1g / kg diet **(T3),** Peppermint 15 g / kg diet **(T4),** or Peppermint 20 g / kg diet **(T5).** five corn-soybean study diets were used through the study period (20-34 weeks). The probiotic used is microbial feed additive (high-activity strain of ***Bacillus subtilis*** (DSM17299) at a minimum concentration of 1.6 x 10^9 viable spores/g), supplied by Biochem. (GalliPro ®). All groups were maintained under the same environmental and managerial conditions. Water and feed were provided ad-labitum. The study period (20-34 weeks of age) divided to three sub periods. During the study periods body weight, egg number and egg weight were recorded. Shape index and yolk index were determined and according Romonoff (1949) as follows: Shape index (%) = (width/length) *100 Yolk index (%) = (height/diameter) *100 Egg shell, thickness, including shell membranes, was measured using amino meter at the equator. The yolk egg visual color score was determined by matching the yolk with one of the15 bands of the Roche Improved yolk color Fan. Were used to calceolate Haugh Units (HU) HU = 100 log (h-1.7 w $^{0.37}$+7.57) according to Haugh (1937) where: **(h)** height of thick albumen mm, (**w)** weight of egg gm.

Sample collection and biochemical analysis:

At the end of the experiment (34 weeks), 5 birds from each treatment were selected randomly for sample collection. For hematological analysis, a blood sample (1 ml) from each bird was collected by heparinized needles through the puncture of the wing vein and then transferred to the sample tubes which were instantly placed on ice in a cool container. Hb were analyzed within 2 h of the sample collection according to the methods of Jain, (1983). For the biochemical analysis, other blood samples were obtained from the same birds into tubes without anticoagulant, which were left to coagulate at room temperature. The samples were then centrifuged for 13 min at 3000×g. The supernatants (serum samples) were collected to Eppendorf tubes and stored at − 20 °C until further analysis. After blood collection, the same birds were then slaughtered as per the recommendation of the institutional committee, eviscerated, and take some carcass measurements and meat samples (Breast muscles) such as fatty acid composition (ω-3 and ω-6) was performed and determined according to AOAC, (2000) and the Malondialdehyde (MDA) level was determined in the breast muscle according to McDonald and

Hultin, (1987). AST and ALT activities were assessed using the methods of Reitman and Frankel (1957). Total proteins and albumin were determined in serum according to the methods of Weichselbaum, (1946) and Bartholomew and Delaney, (1964), respectively; globulin was calculated by the difference between total proteins and albumin. The method of Sánchez-Carbayo *et al.,* (1999) was used to assess serum T_3 and T_4 concentrations using available commercial kits (Byk-Sangtec Diagnostica, Dietzenbach-Germany; Immulite 2000, DPC, LA).

Assessments of antioxidant status:

Total antioxidant capacity (TAC) was assessed in serum following the method of Janaszewska and Bartosz, (2002). Catalase enzyme **(CAT)** and superoxide dismutase enzyme **(SOD)** activities were determined according to the methods of Aebi, (1983) and Sun et al., (1988), respectively.

Statistical analysis:

All data were analyzed by one-way ANOVA using the General Linear Model procedure of SAS (SAS Institute, 2009). Tukey's honestly significant difference test was used to test the significant differences among the mean values. For all analysis, the pooled standard error of the mean (SEM) was listed. The results are presented as means and the significant level for differences was set as $p<0.05$.

Results:

Compared with the control group, the supplemental dietary probiotic or peppermint, significant increased ($P<0.0001$) the body weight, egg weight, and egg number at 22, 28 and 34 weeks of age. Although adding dry peppermint leaves was better in body weight at 28 and 34 weeks of age than adding probiotics, in the same way in Egg Number at 28 and 34 weeks. As for the results listed in Table (3) which are oxidative stress biomarkers and two important types of fatty acids in breast meat (ω-3 and ω-6 % of total fatty acids), which showed a significant improvement ($P<0.0001$) in these measurements in each of the studied nutritional supplements compared to the control, with the exception of ω-3, a decline was observed in T2 compared to other additive treatments. Table 4, which contains some measures of blood components, from which it is clear from it that both nutritional supplements achieved a significant improvement ($P<0.0001$) in these measurements, although this improvement was not significant in the serum ALT and AST activities and there were no differences between the two dietary supplements under study. As for Table (5), which includes some measurements of egg quality and affected by the nutritional parameters under study, we find that all the proposed measurements improved significantly ($P<0.0001$) affected by the use of the supplemental dietary probiotic and Peppermint compared with the control group.

Discussion:

As a general finding of this study, the beneficial effects of probiotic treatment on broiler performance achieved in this trial are in agreement with a large number of studies which have shown positive effects of using different strains and combinations of probitotics (Zulkifli *et al*., 2000, Kabir *et al*., 2004 and Lidija *et al*., 2010), Bacillus species are known to produce several extracellular enzymes including α-amylases and cellulose, which increase nutrient digestibility and absorption, many studies have indicated that *Bacillus* species utilizes several nutrients including D-cellulose, D-fructose, D-galactose, α-D-glucose, lactose, lactulose, maltose (Nelson et al., 2009 and Ali et al., 2017). In order to give the scientific explanation of the different results achieved by using probiotics as growth promoters in broiler feed, most of the authors concluded that the efficacy of probiotic application depends on many factors including species composition of probiotics, administration levels, application methods, overall diet composition, bird age and environmental factors (Patterson and Brukholder, 2003 and Lidija *et al.*, 2010). Numerous reports indicated that addition of probiotics as a feed additive, could regulate the intestinal microflora in order to increase the concentration of the beneficial bacteria such as Lactobacillus ssp. and Streptococcus ssp. and inhibit the reproduction of harmful bacteria in the gut (Li *et al*., 2008). Obtained results confirmed the fact exposed by many researchers that the gastrointestinal tract can adapt and react morphologically to external factors related to dietary changes, i.e. addition of probiotics with its effect, it reduces

intestinal cell damage and the need for cell renewal in the gut and increasing villus height, width, and VH:CD ratio might have beneficial effects on bird's performance (Iji *et al*., 2001, Uśćebrka *et al*., 2005, Žikić *et al*., 2008 and Lidija *et al*., 2010), these changes enhance the absorptive surface area, which is important when alternative growth stimulators are applied, however, shorter villus is associated with the presence of toxins (Awad *et al*., 2006 and Ali *et al*., 2017).

It seems that the positive effect of different levels of peppermint on improving in performance, egg quality and the other physiological parameters refer to medicinal plant supplements such as peppermint are used commonly as dietary additives for humans, they are chosen for their non-toxic chemical composition, relatively low cost and easy availability. Also, over the past few years, medicinal plants and their extracts have been used in animal diets as feed additives in order to improve their performance, health and the quality of their products. This use of aromatic plants is based on their wide range of antimicrobial, antioxidant or even appetite and digestion stimulative properties (Hussain *et al*., 2008 and Sharifi *et al*., 2013), peppermint led to significant improvements in growth, which was the result of increased digestion and utilization of nutrients, and a reduction in the intestinal populations of bacteria, such as Clostridia and *Bifidobacteria*. Also, there was a trend towards increased body weight and the amount of *Bifidobacteria*, and a decrease in the ileal Clostridia count in birds fed with the cumin diet compared to the control diet. The results of the present study are in agreement with the observations made by (Ocak *et al*., 2008 and Sharifi *et al*., 2013). its decreasing effects on gastrointestinal disorders, thus strengthening the digestive system and improving feed efficiency, moreover, the antiseptic property of peppermint prevents harmful bacterial growth in the digestive system that led to better digestion and absorption. The antiseptic property of peppermint results from the presence of menthol (Nematollah *et al*., 2017). Nanekarani *et al*., 2012 and Nematollah *et al*., 2017) proved that peppermint strengthened the stomach and intestinal slow motion because of alpha humlone. It seems that the presence of active compounds such as essential oil in the plant stimulate appetite and improve the digestion and mineral absorption and increase feed efficiency. Oxidative stress biomarkers and another blood parameter were improved by peppermint supplement this is due to the essential oil in the peppermint is effective to set the heart's activity and preventing cardiac complications, It should be noted that peppermint has antioxidant activity and is able to counteract free radicals and oxidative stress, antioxidants have been shown to combat a wide variety of diseases, therefore, peppermint, which possess antioxidant activity, might also have protective effects on chicken and also enhance their quality (Nematollah *et al*., 2017).

it is clear to us that it is possible to suggest some nutritional supplements that can improve the performance and physiological parameters, especially in some local breeds such as Mandara laying hens during the period from 20 to 34 weeks of age in Egypt.

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

Ingredient	%	chemical analysis	%
Yellow Corn	59.70	**Crude protein**	16.0
Soybean meal (44%)	24.02	**Metaboliz able energy, kcal/kg**	2700
Wheat bran	5.40	**Crude fiber**	3.72
Corn oil	1.00	**Available phosphorus**	0.40
Limestone	7.77	**Calcium**	3.30
Di-Calcium Phosphate	1.45	**Lysine**	0.90
NaCl	0.30	**Methionine**	.35
Premix*	0.30	**Methionine +cyctine**	0.62
DL-Methionine	0.06	**Sodium**	0.14

***Vitamins and minerals mixture provide per kilogram of diet vitamin A (as alltransretinyl acetate); 1200 IU; Vitamin E (all racatocopheryl acetate); 10 IU; k3 3mg; Vit.D3, 2200 ICU; Riboflavin, 10 mg; Ca pantothenate, 10 mg; Niacin, 20 mg; ; Vitamin B12, 10mg; Vitamin B6, 1.5 mg; Thiamine (as thiamine mononitrate) ; 2.2 mg; Folic acid, 1 mg; Dbiotin, 50mg. Trace mineral (milligrams per kilogram of diet) Mn, 66; Zn, 50; Fe, 30;Cu, 4; Se, 0.1 and Ethoxyquin 3mg.**

Table 2. Effect of dietary supplementation of probiotic and peppermint dry leaves on productive performance.

Items	Treatments					SEM	*p-Value*
	T1	T2	T3	T4	T5		
Body weight at 20 weeks (g)	1225	1243	1244	1245	1243	21.2	< 0.0001
Body weight at 22 weeks (g)	1366^{b}	1411^{ab}	1447^{ab}	1476^{a}	1490^{a}	29.8	< 0.0001
Body weight at 28 weeks (g)	1563^{c}	1584^{bc}	1644^{b}	1815^{a}	1865^{a}	22.1	< 0.0001
Body weight at 34 weeks (g)	1749^{b}	1781^{b}	1792^{b}	1964^{a}	2005^{a}	26.7	< 0.0001
Egg weight at 22 weeks (g)	41.72^{b}	44.08^{a}	43.63^{a}	43.88^{a}	44.08^{a}	0.79	< 0.0001
Egg weight at 28 weeks (g)	43.33^{c}	45.26^{b}	46.15^{ab}	46.53^{ab}	47.11^{a}	0.43	< 0.0001
Egg weight at 34 weeks (g)	46.68^{c}	48.05^{b}	50.07^{a}	50.20^{a}	50.89^{a}	0.40	< 0.0001
Egg Number at 22 weeks	19.8^{b}	23.2^{a}	24.8^{a}	24.2^{a}	24.8^{a}	0.60	< 0.0001
Egg Number at 28 weeks	27.8^{d}	40.4^{c}	41.4^{bc}	44.6^{a}	46.0^{a}	1.13	< 0.0001
Egg Number at 34 weeks	39.2^{c}	48.2^{b}	48.8^{a}	49.8^{a}	49.6^{a}	0.40	< 0.0001

$^{a, b, c}$ Means within a row with different superscripts are significantly different (P≤ 0.05).

SEM: Standard error of mean.

Table 3. Effect of dietary supplementation of probiotic and Peppermint dry leaves on antioxidant status, ω-3 and ω-6.

Items	Treatments					SEM	*p-Value*
	T1	T2	T3	T4	T5		
TAC (U/mL)	10.50^{b}	12.80^{a}	13.00^{a}	13.01^{a}	13.40^{a}	0.2	< 0.0001
CAT (U/mL)	4.11^{b}	6.00^{a}	6.10^{a}	6.40^{a}	6.60^{a}	0.3	< 0.0001
SOD (U/mL)	141.70^{b}	154.20^{a}	156.40^{a}	157.30^{a}	157.80^{a}	3.2	< 0.0001
MDA (nmol/ml)	6.40^{a}	3.52^{b}	3.46^{b}	3.44^{b}	3.42^{b}	0.3	< 0.0001
ω-3 (% of total fatty acids).	0.09^{c}	0.14^{b}	0.17^{a}	0.19^{a}	0.20^{a}	0.01	< 0.0001
ω-6 (% of total fatty acids).	2.00^{b}	2.03^{ab}	2.04^{a}	2.05^{a}	2.06^{a}	0.01	< 0.0001

$^{a, b, c}$ Means within a row with different superscripts are significantly different (P≤ 0.05).

SEM: Standard error of mean.

Table 4. Effect of dietary supplementation of probiotic and Peppermint dry leaves on some measures of blood components.

Items	Treatments					SEM	*p-Value*
	T1	T2	T3	T4	T5		
Hb concentration (g/dl)	9.94 ^{b}	9.96 ^{a}	10.18 ^{a}	10.00 ^{a}	10.32 ^{a}	0.1	< 0.0001
Tri-iodothyronine T_3 (ng/dl)	3.76 ^{c}	4.12 ^{b}	4.19 ^{b}	4.34 ^{ab}	4.67 ^{a}	0.1	< 0.0001
Thyroxin T_4 (ng/dl)	20.40 ^{b}	23.3 ^{a}	24.10 ^{a}	24.40 ^{a}	24.70 ^{a}	0.5	< 0.0001
Total proteins (g/dl)	3.89 ^{b}	4.47 ^{a}	4.91 ^{a}	4.69 ^{a}	5.11 ^{a}	0.2	< 0.0001
Albumin (g/dl)	1.30 ^{b}	1.64 ^{a}	1.77 ^{a}	1.54 ^{a}	2.03 ^{a}	0.1	< 0.0001
Globulin (g/dl)	2.59 ^{b}	2.83 ^{a}	3.14 ^{a}	3.15 ^{a}	3.08 ^{a}	0.2	< 0.0001
ALT	6.0	7.2	7.2	7.3	7.4	0.6	< 0.0001
AST	50.80	51.60	53.20	52.60	53.40	3.9	< 0.0001

$^{a, b, c}$ Means within a row with different superscripts are significantly different (P≤ 0.05).

SEM: Standard error of mean.

Table 5. Effect of dietary supplementation of probiotic and Peppermint dry leaves on Egg Quality.

Items	Treatments					SEM	*p-Value*
	T1	T2	T3	T4	T5		
Egg weight	48.83^{c}	50.86^{b}	50.99^{b}	51.22^{ab}	52.37^{a}	0.42	< 0.0001
Egg width	39.67^{b}	40.11^{ab}	40.11^{ab}	40.22^{ab}	40.78^{a}	0.28	< 0.0001
Egg length	51.78^{b}	52.22^{ab}	53.00^{ab}	53.44^{ab}	53.78^{a}	0.54	< 0.0001
Yolk diameter	35.67^{b}	36.00^{ab}	36.78^{ab}	37.33^{ab}	37.78^{a}	0.68	< 0.0001
Albumen diameter	58.11^{b}	61.33^{b}	61.67^{b}	68.89^{a}	72.89^{a}	2.20	< 0.0001
Albumen height	7.56^{b}	7.78^{b}	7.78^{b}	8.00^{b}	9.78^{a}	0.33	< 0.0001
Albumen weight	29.95	30.42	31.58	31.70	32.11	0.55	< 0.0001
Yolk height	16.78^{b}	16.89^{b}	17.33^{ab}	17.56^{ab}	18.00^{a}	0.22	< 0.0001
Shell weight	4.79^{c}	4.87^{c}	5.00^{bc}	5.45^{ab}	5.08^{a}	0.19	< 0.0001
Shell thickness	34.00^{c}	35.67^{bc}	36.11^{bc}	36.89^{b}	39.11^{a}	0.74	< 0.0001
Yolk color	6.44^{b}	6.77^{b}	7.77^{a}	7.88^{a}	8.33^{a}	0.33	< 0.0001
Eggs shape	73.86^{b}	75.69^{ab}	76.33^{ab}	76.85^{a}	77.75^{a}	0.6	< 0.0001
% Shell W	9.57^{b}	9.78^{b}	9.81^{b}	10.42^{b}	11.40^{a}	0.25	< 0.0001
% Albumen W	58.87^{b}	60.26^{ab}	62.18^{a}	62.28^{a}	62.61^{a}	0.90	< 0.0001
% Yolk W	27.59	27.90	28.25	29.31	29.71	0.95	< 0.0001
Haugh- Unit	89.17^{b}	90.26^{b}	91.00^{b}	91.58^{b}	99.84^{a}	1.85	< 0.0001

$^{a, b, c}$ Means within a row with different superscripts are significantly different (P≤ 0.05).

SEM: Standard error of mean.

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