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Effect of mentha on performance, haematological and biochemical parameters in laying hens

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Abstract

Effects of *Mentha piperita* (peppermint) extract and juice on performance and immune parameters were evaluated in laying hens. A total of 252 Babcock laying hens were allocated to seven treatments with four replications of nine hens. The control hens were fed a basal diet without supplementation. Other hens were given diets supplemented with mentha extract (ME) at 50 (50ME), 100 (100ME), and 200 (200ME) mg/kg of feed or with 50 mentha juice (50MJ), 100 (100MJ) and 200 (200MJ) mg/L that was provided in the drinking water. No significant differences were detected among treatments in bodyweight, feed intake, egg mass, egg production, eggshell breaking strength, Haugh unit, and haematological and serological parameters. The ratio of gram feed to gram egg mass (feed conversion) was significantly better in the birds that received 100ME and 200ME compared with the control hens. The yolk colour index was higher in mid trial analysis (28th day). Thus, although the ME supplementation had a positive effect on feed conversion ratio and egg yolk colour at dosage rates up to 200 mg/kg, further research is needed to establish the efficacy of this herbal product and to determine the most appropriate amount to include in diets for laying hens.

Keywords: antibiotics, immuno-modulator, mentha juice, phytobiotics
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Introduction

Various types of phytogenic product – such as mentha, garlic, anise, cinnamon, coriander, oregano, chili, pepper, rosemary, rosehip and thyme – may be used as additives to enhance performance and modulate gut health in poultry (Criste *et al*., 2017). Phyto-additives have a positive effect on performance parameters, including egg production, egg quality (Rahimi *et al*., 2011; Khan *et al*., 2012), the immune system and antioxidant status (Gill, 2000; Abd El-Hack & Alagawany, 2015). Phytobiotic products improved performance by increasing the digestibility and retention of nutrients, increasing the secretion of digestive enzymes and mucous production, and improving gut health status and microbial population to maintain production performance during heat stress (Criste *et al*., 2017). Among these, mentha is of great interest because of its health-modulating effects. Dietary supplementation of peppermint leaves by up to 2% in a layer hen diet may enhance the production performance and egg quality and reduce cholesterol level (Abdel-Wareth & Lohakare, 2014). Inclusion of thyme herbal extracts in poultry diets was witnessed to improve performance, immunity and antioxidant activity and biochemical profile (Abd El-Hack & Alagawany, 2015). Supplementation of mint in the feed of laying hens decreased serum cholesterol significantly (Abdel-Wareth & Lohakare 2014; Abo-Ghanima *et al*., 2020) and increased calcium and phosphorous. Peppermint extract reduced the blood urea level, triglyceride level and cholesterol in broilers when fed in drinking water (Roozbeh *et al*., 2013) and had a mild effect on immunity enhancement. Supplementation of mentha extract in drinking water decreased plasma total cholesterol, triglyceride, low density lipoproteins (LDL), and liver synthesis of lipid (Rahim *et al*., 2012). Spearmint *(Mentha spicata)* extract may improve egg production, egg shell thickness and yolk weight, whereas it may decrease serum total cholesterol, triglycerides and LDL concentration in laying hens, but had no effect on the immune system (Behboud *et al*., 2011). Pennyroyal (*Mentha pulegium*) powder and its extract had a positive influence on egg production performance, egg quality, blood biochemical and immunity parameters in laying hens (Paymard *et al*., 2013). To the best of the

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authors' knowledge, few research studies on mentha in broilers have shown its positive effect on performance (Ocak *et al*., 2008; Toghyani *et al*., 2010) and data on the use of mentha are scarce, especially as related to its extract and juice in layer diets. Therefore, this study was designed to investigate the effects of ME and mentha juice on the performance, egg quality traits and biochemical and immunity parameters in laying hens. It was aimed to use ME and juice as safe additives with positive effects on performance and immune status.

Materials and methods

This study was conducted at the experimental animal farm of Afyon Kocatepe University under the project approved by BAPK 15.SAĞ.BİL.23. The Ethics Committee of the Faculty of Veterinary Medicine approved the study (case AKUHADYEK-455.15). The results from this study that pertained to the maintenance of egg quality during storage were presented earlier (Rahman *et al.*, 2017).

Two hundred and fifty-two 21-week old Babcock (Hendrix Genetics, Boxmeer, The Netherlands laying hens were procured for this study. The birds were fed a corn-soybean-based basal diet without mentha supplementation during the adaptation period before the trial (Table 1). The basal diet was formulated according to NRC (1994) recommended allowances for laying hens (Table 2). The trial period was 60 days (four days for adaptation to the treatments and 56 days for data collection. The birds were housed in cages with 16 hours of light and eight hours of darkness. Before starting the study, all birds were vaccinated against Newcastle Disease via their drinking water.

Table 1 Ingredient composition of the basal diet provided to laying hens

Feed ingredients	Inclusion level, %
Corn	52.0
Sunflower meal	8.1
Soybean meal	12.2
Thermally treated full-fat soya	12.0
Limestone	9.0
Meat and bonemeal	3.7
Sunflower oil	1.5
Vitamin-mineral mix[1]	0.25
Methionine	0.15
Salt	0.3
Rovabio[2]	0.1
Phytase supplement, providing 500000 mg phytase per kg diet	0.7

[1] Vitamin A: 12000000 IU, vitamin D_3: 3000000 IU, vitamin E: 35000 IU, vitamin K_3: 3500 IU, vitamin B_1: 2750 IU, vitamin B_2: 5500 IU, nicotinamide: 30000 IU, Ca-D pantothenate: 10000 IU, vitamin B_6: 4000 IU, vitamin B_{12}: 15 IU, folic acid: 1000 IU, D-Biotin: 50 IU, choline chloride: IU, manganese: 80000 mg, Iron: 60000 mg, zinc: 60000 mg, copper: 5000 mg, iodine: 2000 mg, cobalt: 500 mg, selenium: 150 mg, antioxidant: 15000 mg/kg
[2] 10 million IU beta xylanase and 17.5 million IU beta glucanase per kg diet

The birds were randomly allotted to seven treatments with 36 birds in each group, and each group was subdivided into four replicates of nine birds. The control hens were fed a basal diet without supplementation. The other hens were given the basal diet supplemented with ME. Thus 50ME, 100ME and 200ME diets contained ME at 50, 100 and 200 mg/kg of feed. The 50MJ, 100MJ and 200MJ treatments provided 50, 100 and 200 mg/L of mentha in the drinking water. The mentha extract was procured from Ideal Animal Health Technologies, Izmir, Turkey. It was extracted by homogenizing clean fresh mentha plants in a blender and filtering the homogenate. The extract was mixed with the diet each day with sunflower oil to prevent loss or oxidative spoilage. Similarly, the juice was poured into fresh drinking water each day. Feed and drinking water were available ad libitum throughout the trial. Fatty acid methyl esters from the extract and juice were prepared and analysed (Canbay *et al.*, 2011). Samples of the basal diet were analysed to determine their nutrient composition according to AOAC (1990)

Table 2 Nutrient composition of the basal diet for laying hens as analysed and as calculated

Nutrient	Analysed, %	Calculated, %
Dry matter	89.88	88.50
Ash	14.29	13.50
Crude fat	6.85	6.50
Crude fibre	5.30	4.60
Crude protein	18.60	18.00
Starch	28.11	
Sugar	3.75	
Metabolizable energy, Kcal/kg	2721.87	2800.00
Calcium		4.00
Phosphorus		0.44
Sodium		0.17
Nitrogen free extract	44.84	

Average daily feed consumption, egg production, egg mass and feed conversion ratio (FCR) were calculated in all groups. Daily egg mass was calculated by multiplying the total number of eggs produced weekly by the average egg weight and dividing the product by the number of days of collection. Daily feed intake and egg mass were then used to calculate FCR. The bodyweight of each bird was recorded at the start of the trial and at the end of the study (day 56). On days 1, 28, and 56 of the trial, three eggs were collected from each replicate of each treatment (84 eggs per day) to analyse egg breaking strength (Orka egg force reader, EF 0468-2011, Orka Food Technology, Wanchai, Hong Kong), egg weight, egg yolk colour, and Haugh unit (Sanovo Engineering Egg Analyzer, Sanovo Technology Group A/S. Odense, Denmark). These analyses were completed on the same day that the eggs were collected. At the end of the trial, three eggs from each replicate were collected, and the egg yolk was separated and diluted with normal saline to analyse cholesterol levels.

At the end of trial, blood samples were collected from the heart of three live birds in each replicate using a 10-ml syringe. Five ml of blood was drawn into tubes coated with the anticoagulant ethylenediaminetetraacetic acid. Total leukocyte count (TLC), lymphocyte count, neutrophil count, monocyte count, haemoglobin level, haematocrit percentage, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count and mean platelet volume (MPV) were determined for each sample using a blood chemistry analyser (Mindray BC 2800 Vet, Shenzhen, China). An additional 5 ml blood sample was collected in a tube without anticoagulant and centrifuged at 7000 rpm for 10 minutes, after which time the serum was collected and stored at -20 °C until it was analysed for serum glucose, cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, egg cholesterol, calcium and phosphorus level by a completely automated enzyme-linked immunosorbent assay (Elisa) (Chemwell 2910, Awareness Tech. Inc.®, USA; ALT: AL021, BEN S.R.L.®; Italy, AST: AS071, BEN S.R.L.®, Italy). Serum IgG (immunoglobulin G) was also analysed for serum antibody titre against the ND vaccine by the ELISA method.

The PASW statistics software (IBM Inc., Armonk, New York, USA) was used in the statistical evaluation of the data. The Kolmogorov-Smirnov test was used to examine the normality of the distribution of the data. One-way analysis of variance was applied to the replicate means to determine the overall significance of treatment effects. For the independent variables, Kruskal-Wallis K sample test was applied using post-hoc analysis with Dunn-Bonferroni test to assess differences between the treatment means. The criterion $P \leq 0.05$ was accepted as indicating statistical significance.

Results and Discussions

Mentha extract and MJ were characterized for their fatty acid composition (Table 3).

Table 3 Important fatty acid methyl esters in mentha extract and juice

Fatty acid methyl ester	Extract	Juice
Palmitic acid (C16:0)	19.44	24.96
Palmitoleic acid (C16:1)	0.26	0.25
Heptadecanoic acid (C17:0)	0.25	0.25
Stearic acid (C18:0)	56.20	53.11
Oleic acid (18:1n9c)	3.20	2.09
Linoleic acid (C18:2n6c)	4.79	4.06
γ-Linolenic acid (C18:3n6)	8.79	8.81
Eicosanoic acid (C20:0)	1.62	0.51

There was no significant difference ($P > 0.05$) in the bodyweight among the groups during the study (Table 4). Similarly, bodyweight was not affected by supplementation with extracts of cinnamon, capsicum, oregano and thyme (Lippens *et al.*, 2005)**.** However, Popović *et al. (*2016) observed increased bodyweight as a consequence of herbal supplementation.

Table 4 Bodyweight and bodyweight change during the experimental period in which laying hens received supplemental mentha extract or mentha juice

Treatment	Initial bodyweight, g	Final bodyweight, g	Bodyweight change, g
Control	1551.27	1583.93	40.95
50ME*	1514.00	1588.00	84.89
100ME	1555.28	1600.00	44.45
200ME	1518.65	1588.49	71.63
50MJ**	1535.00	1582.76	46.73
100MJ	1553.94	1638.79	86.06
200MJ	1567.35	1624.62	46.97
SE	6.95	7.03	7.64
P-value	0.31	0.20	0.46

50ME, 100ME, 200ME: birds fed diets supplemented with 50, 100 and 200 mg/kg of feed of mentha extract; 50MJ, 100MJ, 200MJ: birds whose drinking water was enriched with 50, 100 and 200 mg/L of mentha

Likewise, no difference ($P > 0.05$) that was attributable to the treatments was observed in the feed intake (Table 5). Thus, these results are contradictory to those findings in which supplementation of laying hens with up to 2% ground peppermint leaves enhanced the feed intake during the late laying period (Abdel-Wareth & Lohakare, 2014). These findings were counter to previous results that showed that the addition of extracts from *Allium sativum*, *Punica granatum* and *Thymus vulgaris* or *Salvia rosmarinus* and *Cinnamomum verum* to drinking water at 0.5 ml/L reduced the intake in laying hens (Sharma *et al*., 2020; Abo-Ghanima *et al*., 2020). The differences between the present study and previous reports might be because of differences in the mechanism of action of the herbal products, the form in which these products were provided, and in the birds that were studied.

Table 5 Weekly average daily feed consumption of laying hens that received diets supplemented with mentha extract or juice

Treatment	Weeks								
	1	2	3	4	5	6	7	8	1 - 8
Control	96.31	101.43	103.62	109.62	109.96	110.51	102.97	104.65	104.16
50ME	96.33	99.50	100.11	104.11	102.47	100.23	99.43	96.11	99.17
100ME	97.12	101.15	105.19	107.01	107.44	107.30	103.47	100.95	102.87
200ME	86.85	99.06	100.15	103.62	107.25	105.01	107.02	101.66	100.64
50MJ	95.69	98.49	103.12	106.73	108.87	108.38	103.54	102.60	102.85
100MJ	94.13	100.71	104.87	108.96	107.61	109.30	108.18	109.29	104.40
200MJ	100.22	100.54	108.03	109.75	107.13	110.58	104.49	109.68	105.69
SE	1.35	0.95	1.07	1.18	1.21	1.30	1.46	1.76	0.98
P-value	0.23	0.99	0.44	0.73	0.82	0.34	0.81	0.43	0.14

50ME, 100ME, 200ME birds fed diets supplemented with 50, 100 and 200 mg/kg of feedof mentha extract; 50MJ, 100MJ, 200MJ birds whose drinking water was enriched with 50, 100 and 200 mg/L of menthe

No differences (*P* >0.05) were detected among the treatments in mean egg mass (Table 6). Contrary to the present results Khan *et al*. (2012), Abdel-Wareth *et al*. (2013), Abdel-Wareth and Lohakare (2014), and Abo-Ghanima *et al*. (2020) claimed that addition of herbs such as thyme, oregano, rosemary, cinnamon essential oils and powdered mint leaves improved egg mass and egg weight for hens that were 68 - 72 weeks old. Mansoub (2011) reported that supplemental thyme improved egg weight and egg production. Similar to the present results, Ghasemi *et al*. (2010) and Sharma *et al*. (2020) reported that supplementation of the diet for laying hens with medicinal herbs (garlic, thyme and essential oils) did not improve egg weight and mass. The present finding of no difference in egg mass is consistent with the absence of a change in the egg weight and egg production. Effects of phytobiotics are commonly thought to vary depending on the type of product, that is, extract, juice or leaf powder, and the amount of bioactive substance being provided to the birds.

Table 6 Weekly egg mass of laying hens that received diets supplemented with mentha extract or juice

Treatment	Weeks								
	1	2	3	4	5	6	7	8	1 - 8
Control	48.00	51.62	52.96	53.00	53.90	57.65	52.49	54.12	52.97
50ME	46.13	51.08	51.22	55.24	56.16	54.76	53.08	54.55	52.78
100ME	50.06	53.36	55.18	54.59	57.86	58.23	54.93	59.63	55.48
200ME	44.49	48.17	50.87	50.32	52.32	53.46	53.55	58.54	51.47
50MJ	44.17	50.14	49.90	51.17	54.58	55.93	56.71	57.92	52.56
100MJ	46.34	47.66	48.72	51.87	53.97	53.79	52.07	54.32	51.09
200MJ	53.11	52.47	54.29	55.95	56.42	58.32	52.57	56.09	55.03
SE	1.13	0.94	0.89	0.83	0.67	0.84	0.61	0.72	0.60
P-value	0.34	0.66	0.44	0.49	0.37	0.56	0.39	0.19	0.39

50ME, 100ME, 200ME birds fed diets supplemented with 50, 100 and 200 mg/kg of feedof mentha extract; 50MJ, 100MJ, 200MJ birds whose drinking water was enriched with 50, 100 and 200 mg/L of mentha, respectively

After feeding supplemental ME for eight weeks, FCR was improved (*P* <0.05) for those birds in 100ME and 200ME relative to the control diet (Table 7). It has been claimed herbal supplements do not affect FCR in laying hens (Christaki *et al.*, 2012; Ghasemi *et al.*, 2010; Abo-Ghanima *et al.*, 2020). However, other studies indicated beneficial effects on FCR from feeding herbal products to laying hens (Bolukbasi & Erhan 2007; Lippens *et al.*, 2005; Rahimi *et al.*, 2011; Abdel-Wareth & Lohakare, 2014; Sharma *et al.*, 2020). The change in FCR at week 8 without contaminant changes being detected in feed intake might have resulted from the numerical increase in egg mass in 100ME and 200ME, despite there being insufficient statistical power to detect these differences.

Table 7 Weekly feed conversion ratios for laying hens that received diets supplemented with mentha extract or juice

Treatment	Weeks								
	1	2	3	4	5	6	7	8	1 - 8
Control	2.01	1.97	1.92	2.00	2.04	1.92	1.96	1.93[ab]	1.97
50ME	2.13	1.96	1.96	1.80	1.82	1.83	1.88	1.76[bc]	1.88
100ME	1.95	1.90	1.93	1.86	1.86	1.84	1.88	1.69[c]	1.86
200ME	1.97	2.06	1.97	1.96	2.08	2.00	2.02	1.74[c]	1.96
50MJ	2.18	1.98	2.08	2.02	2.00	1.94	1.82	1.77[bc]	1.96
100MJ	2.06	2.15	2.16	1.97	2.00	2.04	2.08	2.01[a]	2.05
200MJ	1.89	1.93	1.97	1.91	1.90	1.90	2.00	1.96[a]	1.92
SE	0.04	0.03	0.03	0.03	0.04	0.03	0.04	0.03	0.02
P-value	0.65	0.52	0.46	0.61	0.41	0.60	0.52	<0.01	0.08

[a,b,c] At week 8, means with a common superscript do on differ with probability $P \leq 0.05$
50ME, 100ME, 200ME birds fed diets supplemented with 50, 100, and 200 mg/kg of feedof mentha extract; 50MJ, 100MJ, 200MJ birds whose drinking water was enriched with 50, 100, and 200 mg/L of menthe

There were no detectable differences in egg production percentage, with a slight numerical increase in production percentage in 100ME (Table 8). Similarly, Mahmoud *et al*. (2010) and Ghasemi *et al*. (2010) found that the provision of garlic juice, garlic powder or thyme powder had no effect on egg production in laying hens. However, there are numerous reports of increased egg production resulting from herbal supplementation of laying hens (Bölükbaşi & Erhan, 2007; Rahimi *et al*., 2011; Khan *et al*., 2012; Mansoub, 2011; Sharma *et al*., 2020; Abo-Ghanima *et al*., 2020). Abdel-Wareth and Lohakare (2014) also stated that inclusion of powdered peppermint leaves by up to 2% in the diet of laying hens increased their egg production.

Egg breaking strength (EBS) varied (P <0.05) among the treatment groups at the beginning of the experimental period. This must be the result of the randomization of birds to treatments rather than an effect of the treatments because no differences should exist on day zero because treatments that were only just being applied simultaneously. The egg yolk colour index was higher (P <0.05) in all supplemented groups on day 28 of the trial collection compared with the control group. The Haugh unit remained unchanged during the trial (P >0.05) (Table 9). Khan *et al.* (2012) claimed that the addition of garlic had a positive effect on egg quality parameters. Sharma *et al*. (2020) and Bölükbaşi and Erhan (2007) reported that the addition of herbs and their extracts blended from *Allium sativum*, *Punica granatum* and *Thymus vulgaris* in diets of laying hens increased egg quality parameters, that is, shell thickness, yolk colour and the Haugh unit. Mansoub (2011) found that the addition of thyme in the diets of laying hens enhanced the egg yolk index significantly. Mahmoud *et al*. (2010) and Abo-Ghanima *et al.* (2020) explained similar results of improved HU in administration of phytogenic products (garlic juice, rosemary, and cinnamon essential oils) in laying hen diets. Other researchers (Nichol & Steiner, 2008; Navid *et al*., 2013) claimed that the addition of herbs and their products had no effect on egg quality traits. In other studies, it was determined that the addition of thyme and oregano powder did not affect egg quality parameters (Abdel-Wareth *et al*., 2013; Ghasemi, *et al*., 2010). Saki *et al.* (2014) declared that supplementation of phytogenic feed additives did not change egg quality. Yalcin *et al.* (2006) reported that no significant effects were obtained by supplementation of 5 and 10 g kg-1 garlic powder on the albumen index, eggshell thickness and Haugh unit values of eggs. The positive effect of mentha supplementation on EBS and yolk colour might be because of the enhanced activity of the digestive system resulting in improved utilization of feed nutrients with the help of digestive enzymes boosted by herbal additives.

Table 8 Weekly egg production percentage of laying hens that received diets supplemented with mentha extract or juice

Treatment	Weeks								
	1	2	3	4	5	6	7	8	1 - 8
Control	86.51	89.29	89.47	94.05	93.65	94.49	88.99	90.82	90.91
50ME	82.14	92.06	89.29	93.25	94.44	93.65	88.89	93.25	90.87
100ME	88.10	91.67	93.25	93.25	98.81	97.62	92.63	97.22	94.07
200ME	79.76	80.95	86.11	86.11	87.70	87.30	88.49	93.06	86.19
50MJ	79.37	87.30	87.70	88.89	92.06	92.86	94.84	96.82	89.98
100MJ	82.14	84.52	84.13	86.51	91.27	89.29	88.06	87.50	86.68
200MJ	92.86	90.87	92.54	97.62	98.02	97.12	90.16	89.95	93.82
SE	1.87	1.61	1.45	1.49	1.22	1.20	1.18	1.23	
P-value	0.45	0.51	0.67	0.32	0.18	0.18	0.74	0.30	0.32

50ME, 100ME, 200ME: birds fed diets supplemented with 50, 100 and 200 mg/kg of feedof mentha extract; 50MJ, 100MJ, 200MJ: birds whose drinking water was enriched with 50, 100 and 200 mg/L of mentha, respectively

Table 9 Time course of egg breaking strength, yolk colour index and Haugh unit of laying hens receiving diets supplemented with mentha extract or juice

Treatment	Egg breaking strength			Egg yolk colour			Haugh unit		
Day	0	28	56	0	28	56	0	28	56
Control	45.26[cb]	51.19	55.99	12.00	9.75[b]	11.18	82.68	60.15	63.53
50ME	55.62[a]	47.71	49.60	12.75	12.50[a]	11.67	80.62	68.98	72.48
100ME	41.16[c]	50.59	44.70	11.58	11.75[a]	11.67	83.02	77.66	63.50
200ME	44.55[bc]	49.37	46.72	11.40	12.08[a]	12.09	85.99	65.45	70.15
50MJ	50.79[ab]	52.55	48.68	11.83	11.92[a]	10.42	82.29	68.78	69.52
100MJ	47.26[abc]	51.25	46.83	11.17	11.58[a]	11.92	82.16	58.53	50.40
200MJ	42.50[bc]	50.50	47.96	12.58	11.45[a]	11.67	76.86	58.75	57.43
SE	1.15	0.90	1.02	0.19	0.21	0.19	0.77	2.49	2.75
P-value	<0.01	0.87	0.09	0.21	0.02	0.28	0.09	0.35	0.32

[a,b,c] within a column, means with a common superscript do on differ with probability $P \leq 0.05$
50ME, 100ME, 200ME: birds fed diets supplemented with 50, 100 and 200 mg/kg of feed of mentha extract; 50MJ, 100MJ, 200MJ: birds whose drinking water was enriched with 50, 100 and 200 mg/L of mentha

No effects that could be attributed to the treatments were detected (*P* >0.05) on any of the traits measured by the haematological total profile (Table 10). In contrast, Ghasemi *et al*. (2010) found the addition of 0.2% medicinal herbs in a ration for laying hens increased lymphocytes, whereas other haematological traits remained unchanged. Abo-Ghanima *et al*. (2020) reported that addition of essential oils in laying hen diets increased lymphocytes and monocytes without producing changes in the counts of white blood cells (WBC) or red blood cells (RBC), packed cell volume (PCV) percentage and haemoglobin (Hb) percentage.

Biochemical constituents in serum are closely associated with health status of the birds. In this study, no differences were detected among the treatment groups (*P* >0.05), except that 200ME produced significantly lower IgG values compared with the control (Table 11). In contrast, supplementation of thyme significantly reduced total serum protein when fed at 9 g/kg of feed in layers (Abd El-Hack & Alagawany, 2015). Similarly, total cholesterol and LDL cholesterol of laying hens were reduced significantly with 3 and 6 g/kg feed of thyme (Abd El-Hack & Alagawany, 2015). In the same study, immunity was improved with higher IgG values observed with supplemental thyme (Abd El-Hack & Alagawany, 2015). Similar to the present

Table 10 Haematological profiles of laying hens that were fed peppermint (mentha) extract or juice supplemented diets for 56 days

Treatment	TLC	Lymphocyte count	Neutrophil count	Monocyte count	Red blood cell count	HB, g/dl	Haematocrit, %	MCV	MCH	MCHC	Platelet count	MPV
Control	2.47	1.53	0.56	0.04	2.51	10.26	33.78	110.23	30.71[ab]	30.68	27.23	6.41
50ME	2.78	1.95	0.70	0.04	2.35	10.58	35.77	107.04	30.37[abc]	30.38	26.81	6.43
100ME	2.84	2.02	0.67	0.04	2.49	10.68	35.13	107.80	29.96[abc]	30.49	25.21	6.43
200ME	3.04	2.06	0.71	0.04	2.59	10.79	35.02	112.09	28.76[c]	31.06	26.09	6.52
50MJ	2.97	2.15	0.75	0.04	2.51	10.62	36.13	109.15	30.27[abc]	30.51	26.40	6.50
100MJ	2.99	2.09	0.78	0.04	2.57	10.52	35.54	108.53	29.29[bc]	30.19	26.89	6.60
200MJ	2.56	1.79	0.67	0.04	2.59	10.35	35.86	109.61	31.22[a]	30.97	24.89	6.57
SE	0.09	0.07	0.02	0.00	0.03	0.10	0.33	0.86	0.22	0.10	0.31	0.03
P-value	0.57	0.33	0.10	0.06	0.51	0.86	0.62	0.78	0.05	0.24	0.38	0.65

[a,b,c] within a column, means with a common superscript do on differ with probability $P \leq 0.05$
50ME, 100ME, 200ME birds fed diets supplemented with 50, 100 and 200 mg/kg of feed, respectively, of mentha extract; 50MJ, 100MJ, 200MJ birds whose drinking water was enriched with 50, 100 and 200 mg/L of menthe, respectively; TLC: Total leukocyte count, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, MPV: Mean platelet volume

Table 11 Serum constituents and egg cholesterol of laying hens receiving peppermint (mentha) extract or juice supplemented diets for 56 days

Treatment	Glucose, mg/dl	Cholesterol, mg/dl	HDL, mg/dl	LDL, mg/dl	AST, U/L	ALT, U/L	Total protein, g/dl	IgG, mg/ml	Cholesterol, mg/dl	P, mg/dl	Ca, mg/dl
Control	180.64	72.45	19.73	21.82	196.00	0.87	4.16	4.46[a]	309.10	3.65	17.73
50ME	184.27	80.18	19.42	27.00	174.55	1.04	4.63	4.19[ab]	329.09	4.14	19.08
100ME	185.00	72.20	20.40	20.20	186.30	0.94	4.37	2.70[ab]	362.00	3.59	17.23
200ME	161.64	76.00	17.09	22.09	152.09	0.95	3.60	0.94[b]	350.10	5.31	15.60
50MJ	178.00	71.18	20.09	20.27	178.82	0.84	4.23	2.29[ab]	357.18	3.75	17.13
100MJ	183.00	97.00	20.40	26.10	185.90	2.48	4.64	5.46[ab]	321.91	4.11	18.18
200MJ	175.22	89.89	16.56	25.67	185.22	1.73	4.49	1.65[ab]	284.29	3.96	19.16
SE	3.14	5.22	0.43	1.15	5.73	0.18	0.14	0.63	8.99	0.31	0.55
P value	0.44	0.53	0.08	0.51	0.52	0.14	0.44	0.02	0.28	0.81	0.65

[a,b] within a column, means with a common superscript do on differ with probability $P \leq 0.05$
50ME, 100ME, 200ME birds fed diets supplemented with 50, 100 and 200 mg/kg of feed, respectively, of mentha extract; 50MJ, 100MJ, 200MJ birds whose drinking water was enriched with 50, 100 and 200 mg/L of menthe, respectively; P: phosphorus, Ca: calcium

study, Saki *et al*. (2014) reported that dietary inclusion of phytogenic feed additives did not change the serum cholesterol and triglyceride levels in laying hens. Mansoub (2011) found that supplementation with thyme powder in layer diets did not influence the immunity in laying hens, and it showed a significant reduction in serum total cholesterol concentration, but produced no change in serum HDL, LDL and glucose concentration. Ozek *et al*. (2011) and Bozkurt *et al*. (2012) declared that essential oil mixtures did not improve the antibody titre against ND or infectious bronchitis virus in layers. Ghasemi *et al*. (2010) determined that the inclusion of garlic and thyme powder in laying hen diets had no effect on serological parameters, including cholesterol, TG, LDL, and HDL. Abo-Ghanima *et al*. (2020) reported that the addition of essential oils reduced the serum cholesterol level, AST, and ALT values, whereas it increased the concentration of serum calcium (Ca) and serum phosphorous (P) in laying hens. Abdel-Wareth & Lohakare (2014) found that supplementation of peppermint leaf powder by up to 2% in laying hen diets reduced cholesterol levels, but increased serum total proteins with increasing levels of peppermint leaf powder. The literature showed the interchanging results of phytobiotic supplementation on biochemical parameters, which might be because of variable types of herbs or their products that were used in past studies with various dose levels in different species of birds.

Conclusions

Supplementation of the diets of laying hens with *Mentha piperita* oil at levels providing 100 mg/kg and 200 mg/kg mentha extract had positive effects on feed conversion ratio of laying hens after being fed for 8 weeks in this study. Because the rate of significant effects was approximately equal the expected false positive rate at P =0.05, it is suggested that additional studies should explore the efficacy of this herbal product with a wider range of doses and compare the use of extract versus juice to provide recommendations for its use in diets for laying hens.

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Authors' Contributions

This work was derived from the PhD thesis of AR, who wrote this article too. IB conceived the idea and supervised the thesis and research. EEG analysed the data.

Conflict of Interest Declaration

The authors declare no conflict of interest regarding this article.

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