

EFFECT OF DIFFERENT LEVELS OF BASIL AND PEPPERMINT AN ESSENTIAL OILS ON PRODUCTIVE AND PHYSIOLOGICAL PERFORMANCE OF TWO LINES OF GROWING QUAIL (*COTURNIX COTURNIX JAPONICA*)

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(Received 11 October 2021, Revised 16 December 2020, Accepted 28 December 2020)

ABSTRACT: The present study aimed to evaluate the effect of adding basil (*Ocimum basilicum* L.) and peppermint (*Mentha piperita*) an essential oils (EOs) to the diet on growth performance, carcass characteristics and some hemato-biochemical indices of two lines (Brown and Golden) of growing quail (*Coturnix coturnix*). A total of 360 chicks at one day old; 180 chicks for each of the brown and golden lines were randomly distributed over five treatments for both lines; two replicates for the treatment (18 chicks per replicate) in a (5×2) factorial arrangement. The first treatment was involved feeding a basal diet (BD) and served as a control group. The second and third treatments were involved feeding the BD supplemented with Basil essential oil (BEO) with two levels (250 and 500 mg/kg). The fourth and fifth treatments were involved feeding the BD supplemented with Peppermint essential oil (PEO) with two levels (250 and 500 mg/kg). The results indicated significantly ($p \leq 0.05$) higher final body weight and total body weight gain for dietary treatments supplemented with EOs than the control. Feed intake and feed conversion ratio were improved significantly ($P \leq 0.05$) in comparison with the control and T₂. A significant ($P \leq 0.05$) increase in carcass weight compared to the control. Inclusion of BEO and PEO with (500 mg/kg) caused a significant ($P \leq 0.05$) increase in total protein and globulin. Also, feeding with (250 mg/kg) of BEO led to an increase in the albumin level than control group. Dietary supplemented with (500 mg/kg) of PEO significantly ($P \leq 0.05$) decreased total cholesterol. Addition BEO and PEO caused a significant decrease in the total numbers of *E. coli* bacteria and the total numbers of fungi, meanwhile, there was a significant increase in *Lactobacilli* numbers compared to control and T₂. Regarding the line effect, the results showed significant ($P \leq 0.05$) superiority of the brown quail line over the golden line concerning the final body weight, carcass weight, total giblets, gizzard weight, liver weight, total protein, globulin, and lactic acid bacteria numbers. While the golden line was higher significantly ($P \leq 0.05$) than the brown line in the dressing percentage, total edibles, spleen weight, total numbers of bacteria and fungi and the least number of *E. coli* bacteria compared to the brown line. It would have suggested that the supplementation of growing quail diets with (250 and 500 mg/kg) of basil and peppermint EOs improved productive performance and reduced total numbers of harmful bacteria and the total numbers of fungi.

Key words : Quail, basil-peppermint an essential oils, performance, hemato-biochemical indices, carcass characteristics.

How to cite : Rabia J. Abbas, Sajida A. AlShaheen and Tarek I. Majeed (2021) Effect of different levels of basil and peppermint an essential oils on productive and physiological performance of two lines of growing quail (*Coturnix coturnix japonica*). *Biochem. Cell. Arch.* **21**, 27-37. DocID: https://connectjournals.com/03896.2021.21.27

INTRODUCTION

Use of natural additives such as medicinal plants, their essential oils, or extracts in poultry nutrition as antioxidants are gaining attention to the last few years (Mehri *et al*, 2015a). According to Karaskova *et al* (2015), phytogetic additives present a plausible alternative as they enhance a number of important processes in the animal body. In a recent study, Al-Kelabi *et al* (2019) indicated that the addition of sweet basil plant to the diet improved broiler performance by stimulating synthesis and release of growth hormone. An additional advantage

of essential oils is that they can be administered besides vaccination, given that; the phytogetic does not burden the bird's organism and does not require a waiting period before slaughter, which guarantees food safety (Adaszyńska-Skwirzyńska and Szczerbińska, 2020). Phytogetic feed additives containing essential oils of (*Mentha spicata*, *Mentha piperita*, *Ocimum basilicum*, *Salvia officinalis*, *Rosmarinus officinalis* and *Thymus vulgaris*) were studied by Roldán *et al* (2010) who determined the main constituents of essential oils, as well as, tested their antibacterial activity against pathogenic bacteria (*Escherichia coli*, *Salmonella enteritidis*,

Salmonella typhimurium, *Bacillus subtilis acidophilus* and *Bifidobacterium breve*). In this regard, the essential oil has antimicrobial components and it is effective against a lot of bacteria species. Several studies reported significant antimicrobial and anti-fungal properties of basil (*Ocimum basilicum*) and peppermint (*Mentha* spp.) oil or their leaf methanolic extract (Sokovic *et al*, 2009; Pramila *et al*, 2012; Kamatou *et al*, 2013; Moghtader, 2013; Krishan and Narang, 2014; Bokhari *et al*, 2016; Shalayel *et al*, 2017; ELnaggar and El-Tahawy, 2018; Abdel-Wahab *et al*, 2018). Essential oils (EOs) are one of the most promising alternatives to antibiotics, that used as feed supplements to improve growth performance, the microbial habitat of domestic animals and to manipulate gut functions (Riyazi *et al*, 2015). EOs have great potential and are generally considered natural, less toxic, and free from residues (Zhai *et al*, 2018). According to Brenes and Roura (2010), the EOs enhances the production of digestive secretions, stimulates blood circulation, exerts antioxidant properties, reduces levels of pathogenic bacteria, and may enhance immune status. Besides to EOs antioxidant and antimicrobial activities, Bhavaniramyia *et al* (2019) showed that EOs could be used as alternative preservatives to increase the shelf lives of cereals and crops through enhanced the safety and quality of cereals and food products. As for Zhang *et al* (2014) had considered essential oils as promoters of growth in poultry meals. According to Adaszyńska-Skwirzyńska and Szczerbińska (2020) EOs can stimulate the growth and functioning of the body, which translates into both chicken's health and enhanced production parameters. Previous studies conducted on chickens have shown that essential oils adding to diet (separately or a mixture) has an effect on improving growth performance and has a positive effect on intestinal microbiota (Kirsti *et al*, 2010; Khattak *et al*, 2014). In this context and in a study conducted on rabbits, Zeweil *et al* (2017) explained that the adding of blend essential oil [peppermint oil 200 (mg/kg) and basil oil 200 (mg/kg)] to diet, shows beneficial results on feed intake and feed conversion ratio without having any detrimental effects on growth performance, carcass quality or digestibility. From this given background, the objective of this study was to investigate the effects of essential oils of basil and peppermint on growth performance, carcass characteristics, some blood biochemical parameters, and total bacterial count of two lines of quail (brown and golden).

MATERIALS AND METHODS

Birds and dietary treatments

This experiment was carried out in the Poultry Farm, Department of Animal Production, College of Agriculture,

and University of Basra. The experiment was lasted 42 days start from 24/10/2019 to 5/12/2019. A total of 360 one day old quail chicks (180 chicks evenly for the brown and golden lines), were raised together up to a week old and then the chicks of each line were weighed and assigned at random to five dietary treatments; each of two replicates (cages) (18 birds per replicate) in a 5×2 factorial arrangement. The birds were allowed free access to feed and water and fed the basal diet formulated to meet the nutrient requirements of quail. The following 5 dietary treatments were used: Treatment not supplemented with essential oils (EOs) additive served as control group 1st, while the 2nd and the 3rd treatments were supplemented with 250 and 500 mg/kg basil (*Ocimum basilicum* L.) oil, respectively. The 4th and 5th treatments were supplemented with 250 and 500 mg/kg peppermint (*Mentha piperita*) oil, respectively. Ingredients and chemical analysis of the basal diet are shown in Table 1. The chicks were fed a starter diet until 21 days of age, followed by a grower diet from 22 up to 42 days of age. All chicks were reared under uniform

Table 1 : Ingredients and nutrient composition of quail starter and grower diets.

Ingredient (%)	Starter diet (1-21 days)	Grower diet (22-42 days)
Yellow corn	49.00	55.00
Wheat	8.50	8.50
Soybean meal (44%)	34.00	29.00
¹ protein concentrates (44%)	6.00	5.00
Vegetable oil	1.00	1.00
Dicalcium phosphate	0.25	0.25
Limestone	0.75	0.75
² Mineral premix	0.30	0.30
Sodium chloride	0.20	0.20
Total	100	100
³ Calculated composition		
Metabolizable energy (MJ/kg)	12.23	12.49
Crude protein (%)	23.00	20.83
Crude fat (%)	2.98	3.05
Crude fiber (%)	3.80	3.20
Calcium (%)	0.75	0.69
Phosphorus available (%)	0.36	0.32
Calcium: phosphorous	2.08	2.15
Lysine (%)	1.24	1.09
Methionine + Cysteine (%)	0.79	0.72

¹Protein concentrate used from Al-Hayat Company, Jordanian Origin, to provide the following per kg of diet: 44% protein, 2800 kcal/kg ME, 12% fat, 25% ash, 5% calcium, 2.9% phosphorus, 2.55% methionine + Cysteine, 2.8% lysine. ²Content/kg: Manganese 80 g; iron 80 g; zinc 50 g; copper 10 g; cobalt 2 g; Iodine 1 g; excipient q. s⁻¹, 000 g. ³Was calculated according to the chemical composition of feedstuff contained in NRC (1994).

management conditions throughout the experiment period of 42 days. Live body weight was recorded at the beginning and at the end of the study. Body weight gain was calculated from the difference in body weight for the period (7-42) days of age. Feed consumption was calculated as the difference between the amount of feed offered through the experimental period and the feed residue at day 42 for the experimental period (7-42). Feed efficiency ratio was calculated by determining the amount of feed consumed for the experimental period (7-42) days of age per weight gain for the same period of the experiment.

Carcass and internal organs evaluation

At time of slaughtering (42 days of age), four birds from each line and treatment (2males, 2 female) of similar body weight were individually weighed and slaughtered. Weights of different internal organs (gizzard, liver, heart, spleen, bursa of Fabricius and gut) were recorded. Dressing percentage was calculated by dividing the prepared carcass weight by live weight $\times 100$. Bursa index was calculated by dividing the weight of bursa of Fabricius gland by the gland weight for the control group (Lucio and Hitchner, 1979).

Heamatological biochemical parameters evaluation

At time of slaughtering (42 days of age), four birds from each line and treatment (2chick/replicate) were selected at random and slaughtered to collect blood samples. Red Blood Cells (RBC) and total White Blood Cells (WBC) were measured according to the method of Natt and Herrick (1952). Packed Cell Volumes (PCV) was measured according to Archer (1965). Hemoglobin (Hb) was calculated directly based on PCV values using the equation described by Campbell (1995). For serum biochemical indices, blood sample was drawn and allowed to stand for an hour at room temperature (18°C). Serum was separated by centrifuge and stored at (-20°C) and total protein and albumin were analyzed by a colorimetric method using commercial kits (Spinreact, Spain). Globulin (Gb) concentration was calculated as the difference between total protein and albumin concentrations. Serum glucose and cholesterol concentrations were determined according to the methods of Tietz (1999) using commercial kits (Biolabo AS, France).

Microbiological analyses

The total numbers of microbial groups were estimated according to Petrifilm™ plates method mentioned by Blackburn and McCarthy (2000). Microbial analyses were performed to count the total aerobic bacteria (TBC), total coliforms *Escherichia coli* (*E. coli*) bacteria, lactic acid bacteria (LAB) and total fungi (TFC).

Swab samples of jejunum contents also were collected from both quail lines (four birds per treatment) at 42 days of age, later, tubes of swab samples were transferred to the microbial population laboratory. Dilutions were prepared with Peptone water, then incubated in order to count the numbers of microorganisms using a Petri film plates consists of a flat plate containing a circle divided into twenty small squares. Then, 1 ml of dilution prepared for the implanting transferred by micro-pipette to the Petri film slowly and gently to prevent air bubble or scratches and left for a period to ensure equal spread. All plates were incubated at 37°C for 24 h for bacteria and for a period 48-72 h at 24°C for fungi (Blackburn and McCarthy, 2000).

Statistical analysis

A factorial analysis was conducted to test the main effects (Treat and lines) and their interactions using the SPSS Software (2012) statistics application of the 2-way ANOVA procedure. Differences among means were assessed using the Least Significant Difference (L.S.D.) test at $P \leq 0.05$ (SPSS, 2012).

RESULTS

Growth performance

The effect of dietary supplementation with basil essential oil (BEO) and peppermint essential oil (PEO) on the productive performance of quail chicks is presented in Table 2. Body weight at 7th days of age of chicks did not differ significantly ($p \geq 0.05$) among all the experimental treatments. However, chicks of the brown line showed higher ($p \leq 0.05$) body weights than those of golden line chicks at 7th day of age. The results also revealed that final body weight of quail birds that were fed diet supplemented with BEO and PEO were significantly ($P \leq 0.05$) increased comparing to those of the control group birds (Table 2). Besides that, total weight gain was positively influenced by the dietary treatments. Regarding line effect, final body weight at 42 days of age of brown line was significantly ($P \leq 0.05$) higher compared to that of golden line (188.78 and 177.33 g, respectively).

Data of feed intake and feed conversion ratio in Table 2 reveals significant ($P \leq 0.05$) differences among dietary treatments with respect to feed intake and feed conversion ratio (FCR) for the period (1-6) weeks of the experiment. Basil and peppermint essential oil supplemented treatments showed significantly ($P \leq 0.05$) lower feed intake and better feed conversion ratio than control group and dietary treatment of adding 250 (mg/kg) BEO. The results also revealed significant ($p \leq 0.05$) increase in feed intake for brown line birds compared to the golden, whereas, golden line birds showed better

($p \leq 0.05$) FCR in comparison with brown during the period of 1-6 weeks (Table 2).

Carcass characteristics

The effect of different dietary treatments on carcass and some internal organs is presented in Table 3. There was an increase ($P \leq 0.05$) in carcass weight respecting to BEO or PEO dietary supplementations treatments compared to the control group; meanwhile, there was a significant difference ($P \leq 0.05$) in gizzard weight among all treatments. On the other hand, the dietary supplementation of BEO and PEO did not significantly ($P \leq 0.05$) affect the dressing percentage, total giblets, total edible parts, liver weight, heart weight and gut weight and relative weight of the bursa of fabricius and bursa index.

Haematological and serum biochemical analysis

Hematological measures (hemoglobin (Hb), red blood count (RBC), white blood cell (WBC) and Packed cell volume (PCV), under different basil essential oil (BEO) and peppermint essential oil (PEO) treatments are shown in Table 4. The Hb, RBC and WBC values were similar among different BEO and PEO treatments, while there were significant ($P \leq 0.05$) differences among treatments

in PCV percent. Birds that fed on diets supplemented with (500 mg/kg) BEO showed significantly ($P \leq 0.05$) higher PCV % as compared with other treatments. For serum biochemical indices, there were significant ($P \leq 0.05$) differences in the values of total protein, albumin, globulin and cholesterol, while there were no significant ($P \geq 0.05$) differences in the values of albumin (A) to globulin (G) ratio and glucose levels (Table 5). The results also revealed that supplementation diet with (500 mg/kg) BEO resulted in superior ($P \leq 0.05$) values of total protein and albumin as compared with other treatments. In addition, birds that have been fed diet with (500 mg/kg) PEO had higher ($P \leq 0.05$) globulin values as compared with other treatments. Data of total cholesterol (TC) in Table 5 reveals significant ($P \leq 0.05$) differences among all treatments, PEO supplemented treatments showed significantly ($P \leq 0.05$) lower (TC) than control group. Regarding line effects, the brown line showed higher ($p \leq 0.05$) total protein and globulin concentration in comparison with the golden line.

Microbial analysis

The results in Table 6 showed that there was a significant ($p \leq 0.05$) reduction in total *E. coli* bacteria and total count of fungi, whereas, the *Lactobacilli*

Table 2 : Effect of experimental treatments on performance of quail chicks at overall period 7-42 days old.

Quail performances	Line	Experimental diets					Mean	SEM	P-value*
		Control (mg/kg)	BEO (mg/kg)		PEO (mg/kg)				
		0.0	250	500	250	500			
Initial body weight(g)	B	22.08	21.64	22.14	21.74	21.91	21.90 ^A	0.319	T = 0.851
	G	18.94	18.42	19.13	18.23	17.95	18.53 ^B	0.257	L = <0.001
	Mean	20.51	20.03	20.63	19.98	19.93	20.22	0.434	T × L = 0.975
Final body weight(g)	B	177.33	188.55	191.99	193.32	192.76	188.78 ^A	2.265	T = 0.030
	G	171.14	179.70	178.56	179.19	178.11	177.33 ^B	1.611	L = < 0.001
	Mean	174.23^b	184.13^a	185.28^a	186.26^a	185.43^a	183.06	1.885	T × L = 0.682
Weight Gain (g)	B	155.25	166.91	169.86	171.59	170.85	166.89 ^A	2.339	T = 0.047
	G	152.20	161.28	159.42	160.51	160.16	158.71 ^B	1.753	L = 0.007
	Mean	153.72 ^b	164.10 ^a	164.64 ^a	166.05 ^a	165.50 ^a	162.80	1.704	T × L = 0.773
Feed intake (g)	B	656.87	621.87	593.84	633.96	556.18	612.54 ^A	12.309	T = 0.030
	G	566.95	558.00	493.32	482.93	516.62	523.56 ^B	14.754	L = < 0.001
	Mean	611.91^a	589.93^{ab}	543.58^{bc}	558.44^{bc}	536.40^c	568.10	13.844	T × L = 0.204
Feed conversion ratio (g/g)	B	4.24	3.73	3.50	3.70	3.25	3.67 ^A	0.117	T = 0.026
	G	3.74	3.46	3.10	3.01	3.23	3.30 ^B	0.124	L = 0.017
	Mean	3.99^a	3.59^{ab}	3.30^b	3.35^b	3.24^b	3.50	0.093	T × L = 0.593

^{abc}Means in the same row with no common superscript are different at $p < 0.05$; ^{AB} Means in the same column with no common superscript are different at $p < 0.05$. SEM: Standard error of the mean,

*P-value**; T= Treat effect; L= line effect; T×L= Interaction effect; BEO: basil essential oil; PEO: Peppermint essential oil; B: Brown; G: Golden.

Table 3 : Carcass characteristics and some internal organ at 42 days of the age of Japanese quail received basil and peppermint essential oils in diets.

Quail performances	Line	Experimental diets					Mean	SEM	P-value*
		Control	BEO (mg/kg)		PEO (mg/kg)				
		0.0	250	500	250	500			
Dressing percentage*	B	70.51	66.30	65.89	66.04	67.39	67.22 ^B	1.5351	T= 0.735
	G	69.86	74.09	72.48	73.25	79.46	73.83 ^A	1.3619	L= 0.004
	Mean	70.19	70.20	69.18	69.64	73.42	70.53	1.1430	T×L=0.453
Carcass weight (g)	B	118.08	127.87	124.70	131.44	126.66	125.75 ^A	1.3136	T= 0.002
	G	116.13	118.72	129.73	115.96	125.32	121.17 ^B	1.6290	L= 0.005
	Mean	117.11^b	123.29^a	127.21^a	123.70^a	125.99^a	123.46	1.0940	T×L=0.002
*Total giblets	B	9.29	10.34	10.52	10.12	10.18	10.09 ^A	0.3244	T= 0.381
	G	8.63	7.81	10.13	8.90	8.81	8.86 ^B	0.3184	L= 0.012
	Mean	8.96	9.08	10.33	9.51	9.49	9.47	0.2452	T×L= 0.634
**Total edible parts	B	79.79	76.64	76.41	76.15	77.56	77.31 ^B	1.2945	T= 0.678
	G	78.49	81.91	82.61	82.16	88.27	82.69 ^A	1.3601	L= 0.009
	Mean	79.14	79.27	79.51	79.15	82.91	80.00	1.0221	T×L= 0.420
Liver weight (g)	B	3.20	4.54	4.25	4.25	4.16	4.08 ^A	0.2364	T= 0.493
	G	3.39	3.09	3.99	3.52	3.62	3.52 ^B	0.1817	L= 0.107
	Mean	3.29	3.82	4.12	3.88	3.89	3.80	0.1539	T×L= 0.542
Heart weight (g)	B	1.79	1.71	1.54	1.61	1.74	1.68	0.0519	T= 0.904
	G	1.64	1.55	1.69	1.68	1.57	1.62	0.0395	L= 0.423
	Mean	1.71	1.63	1.61	1.65	1.66	1.65	0.0325	T×L=0.395
Gizzard weight (g)	B	4.29	4.088	4.73	4.26	4.28	4.33 ^A	0.1611	T= 0.027
	G	3.61	3.18	4.46	3.71	3.62	3.71 ^B	0.1596	L= 0.004
	Mean	3.95^{ab}	3.63^b	4.59^a	3.98^{ab}	3.95^{ab}	4.02	0.1223	T×L=0.930
Gut weight(g)	B	8.70	8.04	8.64	8.21	7.70	8.26	0.4562	T= 0.673
	G	9.09	6.93	7.99	5.52	8.54	7.61	0.7373	L= 0.486
	Mean	8.89	7.48	8.31	6.86	8.12	7.93	0.4311	T×L= 0.765
Spleen weight (g)	B	0.170	0.115	0.160	0.150	0.132	0.145	0.015 ^B	T= 0.634
	G	0.307	0.312	0.277	0.312	0.285	0.299	0.021 ^A	L= <0.001
	Mean	0.238	0.213	0.218	0.231	0.208	0.222	0.017	T×L=0.927
Bursa of fabricius weight(%)	B	0.042	0.052	0.060	0.052	0.055	0.052	0.005	T= 0.395
	G	0.040	0.057	0.077	0.075	0.062	0.062	0.006	L= 0.281
	Mean	0.041	0.055	0.068	0.063	0.058	0.057	0.004	T×L=0.913
Bursa index	B	1.000	1.052	0.958	0.990	1.003	1.001	0.017	T= 0.911
	G	1.000	0.986	1.036	1.058	0.991	1.014	0.014	L= 0.570
	Mean	1.000	1.019	0.997	1.024	0.997	1.007	0.011	T×L=0.293

^{abc}Means in the same row with no common superscript are different at ($p \leq 0.05$), * Total giblets : (gizzard+ liver+ heart). ** total edible parts : (dressing + giblets), ^{AB} Means in the same column with no common superscript are different at ($p \leq 0.05$). SEM: Standard error of the mean, P-value *: T= Treat effect, L: line effect, T×L= Interaction effect, BEO: basil essential oil; PEO: Peppermint essential oil; B: Brown; G: Golden.

population was increased significantly ($p \leq 0.05$) in treatments of (BEO and PEO) dietary supplementation as compared to control group. Golden line showed more ($p < 0.05$) total bacterial and total count of fungi in comparison with brown line whereas, the brown line

showed more ($p \leq 0.05$) *Lactobacilli* and *E. coli* population than golden line in the jejunum part of the small intestine. There was a significant interaction between Treatment and Line in numbers of total aerobic bacteria and lactic acid bacteria contents of the jejunum of quail

Table 4 : Effects of basil and peppermint essential oil supplementation on hematological indices of Japanese quail at 42 days of age.

Parameters	Line	Experimental diets					Mean	SEM	P-value*
		Control	BEO (mg/kg)		PEO (mg/kg)				
		0.0	250	500	250	500			
Hb (g/dl)	B	14.63	15.21	14.30	14.55	15.14	14.76	0.2453	T= 0.590
	G	14.52	15.24	14.45	14.94	14.74	14.78	0.1662	L= 0.972
	Mean	14.57	15.22	14.37	14.74	14.94	14.77	0.1442	T×L= 0.960
RBC (×10 ⁶ /mm ³)	B	2.86	2.97	3.12	2.96	2.95	2.97	0.0395	T= 0.464
	G	2.91	2.99	3.01	2.94	2.95	2.96	0.0360	L= 0.859
	Mean	2.88	2.98	3.06	2.95	2.95	2.96	0.0260	T×L= 0.928
WBC (×10 ³ /mm ³)	B	31.62	28.73	31.12	30.17	30.78	30.49	0.4065	T= 0.625
	G	29.40	30.09	29.90	30.33	30.02	29.95	0.3123	L= 0.314
	Mean	30.51	29.41	30.51	30.25	30.40	30.22	0.2568	T×L= 0.287
PCV (%)	B	36.85	38.65	41.37	37.35	38.90	38.62	0.6514	T= 0.184
	G	37.65	37.02	38.28	38.20	38.51	37.93	0.3454	L= 0.314
	Mean	37.25^b	37.84^{ab}	39.82^a	37.78^{ab}	38.71^{ab}	38.28	0.3674	T×L= 0.323

Hb= Haemoglobin concentration, RBC= Red blood cells, WBC= White blood cells, PCV = Packed cell volume. ^{ab} Means in the same row with no common superscript are different at (p≤0.05), ^{AB} Means in the same column with no common superscript are different at (p≤0.05). SEM: Standard error of the mean, *P-value* *: T=Treat effect, L= line effect, T×L=Interaction effect, BEO: basil essential oil; PEO: Peppermint essential oil; B: Brown; G: Golden.

Table 5 : Effects of dietary basil and peppermint an essential oils supplementation on blood biochemical parameters.

Blood parameter	Line	Experimental diets					Mean	SEM	P-value*
		Control	BEO (mg/kg)		PEO (mg/kg)				
		0.0	250	500	250	500			
Total Protein(g/dl)	B	4.19	4.27	4.66	4.32	4.45	4.38 ^A	0.064	T = 0.012
	G	4.09	4.16	4.23	4.19	4.41	4.21 ^B	0.042	L= 0.010
	Mean	4.14^c	4.21^c	4.45^a	4.25^{bc}	4.43^{ab}	4.29	0.041	T × L= 0.198
Albumin(g/dl)	B	1.52	1.64	1.87	1.62	1.69	1.67	0.049	T = 0.099
	G	1.49	1.53	1.62	1.49	1.68	1.56	0.037	L= 0.072
	Mean	1.50^b	1.58^{ab}	1.74^a	1.56^{ab}	1.69^{ab}	1.61	0.033	T × L= 0.655
Globulin (g/dl)	B	2.66	2.62	2.78	2.69	2.75	2.70 ^A	0.022	T = 0.016
	G	2.59	2.62	2.61	2.69	2.72	2.65 ^B	0.019	L= 0.016
	Mean	2.63^{bc}	2.62^c	2.69^a	2.69^{ab}	2.74^a	2.67	0.015	T × L=0.067
A/G Ratio	B	0.57	0.62	0.67	0.60	0.61	0.62	0.016	T= 0.262
	G	0.57	0.58	0.62	0.55	0.62	0.59	0.015	L= 0.235
	Mean	0.57	0.60	0.65	0.58	0.62	0.60	0.011	T×L= 0.867
Cholesterol (mg/dl)	B	186.36	184.21	181.59	182.11	172.71	181.39	1.957	T=0.079
	G	188.31	187.40	180.32	173.31	184.90	182.85	2.180	L= 0.531
	Mean	187.33^a	185.80^{ab}	180.96^{abc}	177.71^c	178.80^{bc}	182.12	1.435	T×L=0.130
Glucose (mg/dl)	B	182.24	176.08	178.34	174.74	176.08	177.49	1.371	T= 0.468
	G	177.13	180.66	178.07	172.13	175.66	176.73	1.614	L= 0.740
	Mean	179.68	178.37	178.20	173.43	175.87	177.11	1.034	T×L= 0.733

^{abc} Means in the same row with no common superscript are different at p < 0.05, ^{AB} Means in the same column with no common superscript are different at p < 0.05. SEM: Standard error of the mean, *P-value* *: T= Treat effect, L= line effect, T×L= Interaction effect, BEO: basil essential oil; PEO: Peppermint essential oil; B: Brown; G: Golden.

Table 6 : The effects of basil and peppermint essential oil supplementation on the microbial population of the jejunum.

Bacteria and Fungi counts	Line	Experimental diets					Mean	SEM	P-value*
		Control	BEO (mg/kg)		PEO (mg/kg)				
		0.0	250	500	250	500			
Total bacterial count ($\times 10^3$)	B	5.35	5.31	5.31	5.31	5.29	^B 5.31	0.0075	T= <0.001
	G	5.44	5.44	5.28	5.31	5.21	^A 5.34	0.0306	L= <0.001
	Mean	5.40^a	5.38^a	5.29^b	5.31^b	5.25^c	5.33	0.0156	T×L= 0.001
Lactic acidbacteria ($\times 10^3$)	B	4.34	4.49	4.45	4.53	4.56	4.47 ^A	0.0267	T= <0.001
	G	4.10	4.17	4.30	4.46	4.46	4.30 ^B	0.0512	L=< 0.001
	Mean	4.22^c	4.33^b	4.37^b	4.49^a	4.51^a	4.38	0.0344	T×L= 0.026
<i>Escherichia coli</i> (<i>E. coli</i>) ($\times 10^3$)	B	4.44	4.34	4.33	4.28	4.29	4.33 ^A	0.0206	T= 0.047
	G	4.13	4.10	3.81	3.65	3.57	^B 3.85	0.0844	L= 0.007
	Mean	4.29^a	4.22^{ab}	4.07^{bc}	3.96^c	3.93^c	4.09	0.0694	T×L= 0.773
Total fungi counts ($\times 10^3$)	B	4.64	4.48	4.32	4.39	4.29	4.42 ^B	0.0448	T= <0.001
	G	4.80	4.79	4.60	4.57	4.36	4.62 ^A	0.0560	L= <0.001
	Mean	4.72^a	4.64^a	4.46^b	4.48^b	3.93^c	4.52	0.0418	T×L= 0.123

^{abc}Means in the same row with no common superscript are different at ($p \leq 0.05$), ^{AB} Means in the same column with no common superscript are different at ($p \leq 0.05$). SEM: Standard error of the mean, *P-value**: T= Treat effect, L: line effect, T×L= Interaction effect, BEO: basil essential oil; PEO: Peppermint essential oil; B: Brown; G: Golden.

(Table 6).

DISCUSSION

Previous studies in broilers have found positive effect of BEO and PEO on body weight and body weight gain, which in line with our findings in broilers (Kirsti *et al*, 2010; Khattak *et al*, 2014; Riyazi *et al*, 2015; Gurbuz and Ismael, 2016; Witkowska *et al*, 2019) and in quails (Benchaar *et al*, 2007). In this context, Arab Ameri *et al* (2016) reported that broiler chickens treated by 1% peppermint powder showed lower average daily gain (ADG), while 2% peppermint powder showed higher ADG at 21 days of age when compared to chicks that were fed the basal diet. In contrast with our results, Akbari and Torki (2014) reported that average body weight and average daily gain of female broiler chicks were not significantly affected by dietary supplementation of peppermint essential oil under heat stress condition. The improvement in body weight and weight gains for supplementary treatments may be due to the impact of different varieties of active components that peppermint contains which affect the process of digestion and stimulate saliva secretion and enhance bile acid synthesis in liver and bile excretion which affect process digestion and lipid absorption (Frankic *et al*, 2009). Besides, in an earlier study, Lee *et al* (2003) reported that improved growth performance in broilers during the grower and finisher phase could be attributed to the presence of essential oils in the diet, which encourages secretions of endogenous digestive enzymes, which then enhance

nutrient digestion and gut passage rate in chickens. Regarding line effects, higher body weight was observed in the brown line than the golden line at 42 days, which might be attributable to higher rates of body weight gain of this line which interfered with effect of dietary oils supplementation, leading to increase utilization of food and improve feed conversion. Our findings are in line with those of Aljumaily (2011), who reported that brown quails had higher body weight than white strain at 2-5 weeks of age. Similarly, Jatoi *et al* (2013) pointed out that imported flock of Japanese quails attained significantly higher body weight than all local flocks. As well as, Inci *et al* (2015), indicated higher live body weights were obtained respecting the wild type of quail than other studied genotypes (white, dark brown and golden). Likewise, Krawczyk and Koseniuk (2020) indicated that Rhode Island Red (R-11) cockerels exhibit a better rate of weight gain compared to the Yellowleg Partridge (-33) breeds. The supplementation of basil and peppermint powder (leaf or seed), essential oil or plant extracts to poultry diets were positively influenced feed consumption and feed conversion ratio (Al-Kassie, 2010; Abbas, 2010; Gurbuz and Ismael, 2016; Zhang *et al*, 2014; Riyazi *et al*, 2015). However, Amasaib *et al* (2013) reported that supplementary of spearmint (*Mentha spicata*) to broilers basal diet showed no significant differences on feed intake and FCR. In respect to genotype effect of line on feed intake and FCR, significant variation among strains in feed intake has been indicated by Akram *et al* (2014).

Inci *et al* (2015), recorded that wild-type group had the highest feed intake and the lowest FCR value than other groups (white, dark brown and golden). Additionally, Jatoi *et al* (2015a) pointed out that the feed intake of four strains designating as M, K, S and Z of Japanese quail were significantly affected during the entire study period (six-week), whereas, feed conversion ratio at week-4 only, and that imported quail strain (M) performed better performance than other strains (K, S and Z). Also, Iqbal (2011) stated that a significant differences in four varieties of Aseel chicken at 1st, 5th, 6th, 7th, 8th, 9th, 10th, 11th, 12th, 13th, 14th and 15th weeks of age. Similarly, significant strain variation in feed efficiency has been pointed at week-4 during the study period (Akram *et al*, 2014). Concerning the effect of basil and peppermint essential oils on quail carcass characteristics, basil essential oil did not affect internal edible organ weights (liver and heart) and carcass yields of Japanese quail at 42 days, this result was similar to results reported by Riyazi *et al* (2015). Besides, Hasan and Sadeq (2020) reported that different peppermint additives (0.5 and 1% peppermint in feed, 0.5 and 1% peppermint in water) did not affect internal organs percentages of broilers at 35 days. A comparable finding was observed by Abdel-Wahab *et al* (2018) in quails. On the other hand, in rabbits the percentages of the cold and hot carcasses, the total edible and non-edible parts, the liver and giblets did not change significantly with inclusion different levels (400 mg of peppermint, 400 mg of basil essential oil, and 200 mg of peppermint plus 200 mg of basil essential oil/kg diet) (Morshedy *et al*, 2019). Contrary to the current findings, dietary supplementation of peppermint showed a decreasing trend in carcass percent of growing Japanese quails (Mehri *et al*, 2015a). Another study stated that the relative percentage of liver weight in the broilers group fed with dry peppermint (*Mentha piperita* L.) was smaller than control (Al-Kassie, 2010). Regarding the line effect, the results showed the superiority ($P \leq 0.05$) of the brown line on golden in the weight of carcasses, total giblets, gizzard, and liver. While the golden line was higher than the brown in the dressing percentage, total edible parts and spleen weight (Table 4). Concerning the effect of genotype on carcass weight, carcass yield, and in accordance with the present results, Inci *et al* (2015) found that wild-type birds of quail surpassed significantly ($p \leq 0.01$) other studied genotypes (white, dark brown and golden quail). Jatoi *et al* (2015b) stated that Mushki variety of Aseel exhibited better carcass and organ development than other varieties (Mianwali, Peshawari and Lakha). In addition, Hussien *et al* (2020) revealed that the light brown (L) line had the best carcass characteristics as compared with white (W)

and dark brown (D) of quail lines. Rhode Island Red (R-11) cockerels are characterized by significantly higher rate of carcass yield without giblets at 20 weeks than the Yellowleg Partridge (-33), according to Krawczyk and Koseniuk (2020), with non-significant differences in this trait at 16 weeks of age. The results of haematological parameters after dietary supplementation with different levels of BEO and PEO (Table 4), revealed no significant ($P \geq 0.05$) differences due to dietary supplement and lines effects on the haematological parameters (Hb, RBC and WBC), while there was significantly ($P \leq 0.05$) difference in packed cell volume (PCV) value at 42 days of age. In addition, basil and peppermint essential oils were significantly impacting on the serum content of total protein, albumin, globulin, and total cholesterol, while glucose and albumin: globulin (A: G) ratio was not affected by these supplements (Table 5). In this regard, Akbari and Torki (2014) stated that peppermint essential oil and chromium picolinate improved the serum content of albumin under heat-stress conditions in broilers. Contrary to the current findings, ELnaggar and El-Tahawy (2018) reported that RBC count, hemoglobin, glucose concentration increased significantly when broiler fed basal diet supplemented with sweet basil, thyme and their oils. As, for total cholesterol, our results support work done by other authors that reported significantly lower cholesterol in broilers (Abbas, 2010; Akbari and Torki, 2014; ELnaggar and El-Tahawy, 2018) and in Japanese quails (Mehri *et al*, 2015b; Abdel-Wahab *et al*, 2018), when the birds received sweet basil and peppermint (*Mentha piperita* L) in their diets. Differently from the present study, Toghiani *et al* (2010) showed that dietary supplementation with peppermint had no effect on total protein, albumin and total cholesterol concentration determined in broilers plasma. Riyazi *et al* (2015) reported that feeding dietary basil essential oil (200, 400, and 600 ppm) did not significantly influence the plasma cholesterol, LDL and HDL levels in broilers. Furthermore, the dietary inclusion of 400 mg of BEO, 400 mg of PEO, and 200 mg of BEO plus 200 mg of PEO/kg diet did not significantly affect the blood parameters (Hb, PCV, RBC, and WBC) and serum cholesterol as compared with control (Morshedy *et al*, 2019). More recently, Hasan and Sadeq (2020) indicated that serum glucose, total protein, and cholesterol of broiler chicks had not affected by peppermint supplementation either in water or feed at 42 days of age. The reduction in the levels of serum total cholesterol when the quails supplemented with PEO may be related to the inhibitory effect of phytobiotics on hepatic 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) and hepatic reductase that regulates the synthesis of

cholesterol in the liver (Ameri *et al*, 2016). The authors explained that the reasons beyond the decrease in TC is the presence of phenolic compounds in peppermint extract and presence of volatile phenolic compounds such as essential oils: Menthol, menthone, menthyl acetate, menthofuran, limonene, polygen, cineole, and azolen. Another study concluded that the reduction in blood cholesterol partly might be related to the alteration of the intestine microbial environment, which increases lactic acid bacteria that may provide an acidic environment for intestine, resulting in decreasing lipid absorption in the gut (Ghazaghi *et al*, 2014). A previous study reported that active ingredients in peppermint extract can prevent or decline the absorption of cholesterol by the intestines, resulting in lowering the serum cholesterol and serum fat (Crossland, 1980). The results also revealed significant ($P \leq 0.05$) proceeding for brown line compared to golden in blood total protein and globulin. In this regard, a previous study on an indigenous and five chicken breeds: viz. Cobb 500, cockerel, Fayoumi (FAY), Rhode Island Red (RIR), and Sonali (derived from RIR cock \times FAY hen), demonstrated that the hemato-biochemical parameters (RBC, Hb, PCV, cholesterol, creatinine, glucose and urea) significantly differs among the breeds (Dutta *et al*, 2013). However, a study with laying hens, Khawaja *et al* (2013) found that different genotypes of crossbred chickens (Rhode Island Red male \times Fayoumi female (RIFI), Fayoumi male \times Rhode Island Red female (FIRI), and White Leghorn male \times FIRI female (RLH) had no significant ($P \geq 0.05$) effect on blood total protein, glucose, cholesterol, and triglyceride values. Many studies indicate that medicinal plants had a significant effect on the microbial population. Regarding the effect of essential oils on the antimicrobial and antifungal activity, the present results (Table 6) in the line with those obtained by Pramila *et al* (2012), who indicated that methanolic leaf extract of mint (*Mentha piperita*) showed antimicrobial activity against clinical isolates of *Escherichia coli*, *Acinetobacter*, *Staphylococcus aureus* and two fungi such as *Candida albicans*, *Candida glabrata*. As well as, P³uchtov^á *et al* (2018) indicated that mint essential oil shows antibacterial activity against seven microorganisms (*Enterobacter cloacae*, *Salmonella* spp., *Klebsiella pneumoniae*, *Escherichia coli* and *E. coli* (ATCC 25922), *Staphylococcus aureus* and *Streptococcus pyogenes*). Additionally, the present results are coincided with, Mehri *et al* (2015a); Abdel-Wahab *et al* (2018), who showed that peppermint (dried leaves) supplementation to quail diets, decreased the number of the harmful *E. coli* bacteria and increased the number of beneficial *Lactobacillus* bacteria. In this

regard, Bento *et al* (2013) pointed that the growth of Gram-negative bacteria like *Escherichia coli* could be inhibited by essential oils. Moreover, Ghazaghi *et al* (2014) stated that carvone, as the main constituent of essential oils in spearmint substantially reduces the colony-forming unit (CFU) of Gram-negative bacteria (i.e. coliforms) but increased the beneficial bacteria (*Lactobacillus*). Recently, Kang *et al* (2019) reported that peppermint essential oil had antibacterial activity against *Staphylococcus aureus* (*S. aureus*) and prolong the shelf-life of food. The author has explained this to the role of PEO that can damage the cell membrane of *S. aureus*, had potent inhibitory effect on *S. aureus* biofilm formation and inactivated and removed the mature biofilm formed by *S. aureus*. The reduction of total bacteria and *E-coli* count by the mint essential oil may be due to the presence of tannins and flavonoids in the plant extract, which could be responsible for the antimicrobial agents (Pramila *et al*, 2012). In this regards, Kaur *et al* (2010) reported that there is a correlative relationship between the phytochemicals such as tannins and flavonoids and the free radical scavenging activity and antibacterial activity. Regarding to peppermint oil antifungal activities, Moghaddam *et al* (2013), reported that the inhibition of the growth of fungal pathogens may be caused by emulsion damage of the cell wall and cell membrane to various degrees due to different capacities of the oil to penetrate the chitin-based cell walls of fungal hyphae. On other hand, Pham *et al* (2020) suggested that adding blend of encapsulated essential oils and organic acids (BLJ) effectively controlled necrotic enteritis (NE) infections after experimental *Eimeria* and *Clostridium perfringens* coinfection in broiler chickens, the author displayed that the BLJ supplementation improved growth performance and gut health in NE-infected broiler chickens by strengthening the intestinal barrier function, positively modulating the gut microbiota community and differentially regulating intestinal immune responses. Differently from the present study, Jang *et al* (2007) stated no significant effects of essential oils on the *Lactobacilli* population in the gut of broiler chicks.

Statistical results did not indicate any evidence of Treat-lines treatment interaction in initial and final body weight, total weight gains, feed intake, feed conversion ratio, *E. coli* and total fungi counts at 7-42 days of age.

CONCLUSION

It could be concluded from the result of this study that supplementary basil and peppermint essential oil at a level 250 and 500 (mg/kg) has a beneficial effect on a productive trait, with no significant effect on the weight of internal viscera, also BEO and PEO in same levels

reduced the serum total cholesterol level, and number of harmful bacteria and total fungi in Japanese quail. Furthermore, the Brown quail line performed better in most of the studied traits than that of the golden line of quails.

ACKNOWLEDGMENTS

Authors gratefully thank staff of Poultry Farm for their assistance in carrying out of this experiment.

Conflicts of interest

The authors declare no conflicts of interest.

Funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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