

EFFECT OF PEPPERMINT (*MENTHA PIPERITA*) POWDER ON PERFORMANCE, GUT MORPHOLOGY AND IMMUNE ORGANS RESPONSE OF COCCIDIOSIS INFECTED BROILERS

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ABSTRACT

This experiment was performed to find out the effect of peppermint on the performance, gut morphology and immune organs of coccidiosis challenged broilers. A feeding trail was conducted on 320-day-old Ross 308 chicks allocated to 32 floor pens in a 2x4 factorial arrangement. The studied factors were: challenge (Negative or Positive) and feed additive (Control, Peppermint and). On day 8 of age, birds in challenged groups were orally inoculated with *Eimeria tenella*. On day 35 *Eimeria* decreased weight ($P < .0001$) gain and deteriorated feed conversion ratio ($P < 0.05$). On 24 and 35 days of age, broilers given salinomycin and peppermint had improved weight gain and feed conversion ratio compared to controls ($P < 0.05$). Significant challenge \times additive interactions were observed for weight gain, feed intake, feed conversion ratio villus height, crypt depth and apparent villus surface area on d 24 and d 35. Challenged broilers in salinomycin and peppermint groups had significantly increased villus height and villus height/crypt depth and decreased crypt depth compared to positive control. Challenged birds exhibited significantly longer, wider and larger area of bursa of Fabricius lymphoid follicles compared to non-challenged birds. These results illustrated that, peppermint has high protective efficacy against declining in the performance and gut health of coccidiosis infected broilers.

Key words: peppermint, broiler, performance, gut morphology, coccidiosis

حسين

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تأثير اضافة مسحوق النعناع على الاداء الانتاجي، الصفات النسيجية للامعاء و استجابة الغدد المناعية لفروج اللحم المعرضة

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المستخلص

اجريت هذه الدراسة لمعرفة مدى تأثير النعناع على الاداء الانتاجي، الصفات النسيجية و الغدد المناعية لفروج اللحم المعرضة للكوكسيديا. استعملت في هذه التجربة مجموع 320 فرخة بعمر يوم واحد من سلالة (Ross 308) و وزعت عشوائياً على (32) قفص ارضي في تجربة عاملية (2x4). العوامل المدروسة كانت: التعرض للكوكسيديا (سالب او موجب) و اضافة علفية (مجموعة السيطرة، اضافة مسحوق النعناع و ساليونومايسين). تم حقن الافراخ بلايميريا تنلا بعمر ثمانية ايام. اظهرت النتائج بأن في يوم 35 من العمر، الايميريا معنويا انخفضت الزيادة الوزنية و تردأت كفاءة تحويل الغذاء. في يومي 24 و 35 من العمر، تحسنت الزيادة الوزنية و كفاءة تحويل الغذاء لدى الفراريج التي غذيت على الساليونومايسين و النعناع مقارنةً مع مجموعة السيطرة. ايضاً التداخل بين عوامل التجربة كانت معنوية من حيث الزيادة الوزنية، كفاءة تحويل الغذاء، طول الزغابات، عمق الخبايا و المساحة السطحية للزغابات. اضافةً الى ذلك، ازدادت طول الزغابات و نسبة طول الزغابات الى عمق الخبايا و انخفضت عمق الخبايا لدى الفراريج المعرضة للايميريا و المغذاة على الساليونومايسين و النعناع مقارنةً مع مجموعة السيطرة الموجبة. ايضاً كانت مساحة، طول و عرض الجريبات اللمفاوية للبورصا زائدة معنوياً في الفراريج المعرضة للكوكسيديا مقارنةً مع الطيور الغير معرضة. اتضحت هذه النتائج بان للنعناع فعالية عالية ضد تدهور الاداء الانتاجي و صحة امعاء الفراريج المعرضة للكوكسيديا.

كلمات مفتاحية: النعناع، فروج اللحم، الاداء الانتاجي، الصفات النسيجية للامعاء، كوكسيديا.

INTRODUCTION

Coccidiosis (*Eimeria* spp.) is one of the several contagious disease caused by protozoan parasite of the genus *Eimeria* (39). It possess a universal importance in poultry production field. The disease may infect all species of poultry in any system of rearing. Globally, the poultry industry has spent a huge amount of money to prevent and treat this infectious disease (36). Numerous species of *Eimeria* have been documented and each of them infects a particular part of birds' intestine. The parasite is multiplying in the intestine of birds causing tissue damage and interruption of gut integrity, resulting in declined feed and water intake and nutrient absorption, increased intestinal passage time, villus atrophy, dehydration, blood loss, and rise sensibility to other diseases (41). Coccidia has also caused a high economic lose in poultry sector through decreasing of birds' body weight gain, poor feed conversion ratio, increasing of morbidity and mortality rate among them, and high medications cost (12). Various pathogens such as *Clostridium perfringens* might be colonized in the intestine which will lead to necrotic enteritis as a result of changes in its function and induced damage of its tissues (24). Caecal coccidiosis caused by *Eimeria tenella* may contributes to an increased severity of blackhead disease in chickens (25). Since 1940s, chemotherapeutic anticoccidials and vaccines have been used as major components in controlling of coccidiosis (11). These compounds, are efficient disease controllers if used in carefully designed prophylactic treatment programs. However, the in-feed using of anticoccidials has been banned universally due to inevitable development of drug resistance to chemical types of anticoccidials by *Eimeria* strains as well as the public concern over the use of chemical anticoccidials in the diet of food animals (1). This has forced the researchers to re-examine alternative type of coccidial control. Mannan oligosaccharide (MOS), n-3 polyunsaturated fatty acids, yeast cell wall extract, medicinal plants and natural herbs and their essential oils all have potential coccidiostat activity and suppressing ability of chickens' and turkeys' intestinal mucosa pathogens (3, 8, 30). The mechanism of action

of medical plants and their extracts might be through their contribution in the stabilization of beneficial microflora and prevention of pathogens colonization in the gut of animals as well as improvement of digestive enzymes secretion and activity (23). Peppermint as a member of *Labiata* family is considered to be used widely in the world as a therapeutic herb (37). Peppermint's active compound like menthol, menthone, carvacrol, limonene, 1, 8-cineol, cineol and α -pinene play an important role as antimicrobials in inhibition the growth of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritidis*, and *Candida albicans* and as a coccidiostat targeting different life cycle stages of *Eimeria* species and subsequent improvement of poultry health and performance (31, 38). Thus the current study was aimed to determine the effect of powder peppermint (*Mentha piperita*) on the performance gut health, immune organs development, and serum biochemical parameters of coccidiosis challenged broilers.

MATERIALS AND METHODS

Birds, diets, and husbandry

A total of 320 d-old Ross 308 chicks were obtained from a private hatchery in Erbil province, Kurdistan region of Iraq. Birds were allocated to 32 floor pens in two separate rooms in the Animal production farm, College of Agricultural Engineering Sciences, University of Duhok. In a 2x4 factorial arrangement within Completely Randomized Design, birds were randomly weighed and assigned to 8 treatment groups of 4 replicates each, with 10 chicks per replicate. The four non challenged (NC) dietary treatments were: negative control (basal starter, grower and finisher diets with no additive), anticoccidial (basal diets supplemented with salinomycin at 60 mg/kg), peppermint 1 (basal diets supplemented with 5 g/kg (0.5%) peppermint powder), and peppermint 2 (basal diets supplemented with 10 g/kg (1%) peppermint powder). The remaining four coccidiosis challenged (C) dietary treatments were [positive control (basal starter, grower and finisher diets with no additive and challenged with *Eimeria*), anticoccidial (basal diets supplemented with salinomycin at 60 mg/kg and challenged with *Eimeria*), peppermint 1 (basal diets supplemented with 5 g/kg

peppermint powder and challenged with *Eimeria*), and peppermint 2 (basal diets supplemented with 10 g/kg peppermint powder and challenged with *Eimeria*). Non-challenged birds were reared under sanitary conditions in the separate room to prevent the cross contamination with *Eimeria*. Birds were fed on starter diets from d 0 to 10, grower diets from d 11 to 24, and finisher diets from d 25 to 35 of experiment. Three basal diets were formulated according to the nutrient specifications of Ross 308 broilers (Table 1). The used peppermint powder was analyzed and its main active compounds show in Table 2. Birds were reared in floor pens (wire mesh

partitioned at 100 × 100 cm) bedded with wood shavings. Temperature and lighting were set in accordance to management guidelines of the Ross 308 broiler strain. Birds were fed and watered *ad libitum*. On d 10, 24 and 35 of age, the remaining feed in the feeders and birds were weighed to measure the major determinants of performance including accumulative weight gain of birds, feed intake and feed conversion ratio of each pen. The experimental procedures were approved by Animal Ethics Committee at Department of Animal Production, College of Agricultural Engineering Sciences, University of Duhok under the approved number: AEC120120201.

Table1. Ingredients and composition of the basal starter, grower and finisher diets as percentage.

Ingredients kg	Starter	Grower	Finisher
Corn	53.06	56.9	61.74
Soybean meal	31.48	32.89	28.23
Fish meal	4	-	-
Vegetable oil	3	4.48	4.57
Limestone	2	1.39	1.35
Dical Phos	2.72	0.95	0.82
Salt	0.11	0.19	0.08
D,L-methionine	0.38	0.32	0.24
L-lysine HCl	0.5	0.26	0.21
L-threonine	0.25	0.13	0.09
Broiler premix	2.5	2.5	2.5
Nutrient composition			
ME (kcal/kg)	3000	3150	3200
Crude protein	23	21	19.16
Crude fiber	2.25	2.38	2.33
Digestible Arginine	1.29	1.14	0.99
Digestible Lysine	1.29	1.14	0.99
Digestible Meth+Cyst	0.87	0.84	0.73
Digestible Tryptophan	0.226	0.24	0.21
Digestible Isoleucine	0.87	0.81	0.73
Digestible Threonine	0.82	0.73	0.63
Digestible Valine	0.99	0.92	0.83
Calcium	1.60	0.9	0.85
Available Phosphorus	0.844	0.450	0.42
Sodium	0.16	0.160	0.16
Chloride	0.35	0.312	0.23
Linoleic	2.18	2.64	2.73

Coccidia challenging

The coccidia (*Eimeria tenella*) used in the present experiment was isolated in the laboratory of Animal Production Department, College of Agricultural Engineering Sciences, University of Duhok. Fresh bloody droppings were collected from infected birds in farms of the department. Samples of bloody droppings were transferred into 2 ml Eppendorf tube then centrifuged at 6000 X g for 5 minutes and supernatant was discarded. The oocysts were allowed to sporulate in 2.5 % (w/v) potassium

dichromate solution for three days at 27 °C. On day 8, challenged birds were inoculated with 5000 sporulated oocysts of *Eimeria*. Unchallenged birds were given 1 mL of 1% (w/v) sterile saline.

Sample collection

On day 24, two birds per replicate were randomly selected, weighed, and euthanized by cervical dislocation. Then, the birds were dissected to remove the small intestine. Weight of small intestine, gizzard and heart were recorded. The weights of immune organs

(liver, spleen, bursa of Fabricius and) were also recorded and calculated as mass per unit of live body weight (g/100 of live body weight). Approximately 1 cm of the jejunum and whole bursa of Fabricius from each of two bird per replicate were sampled. The intestinal

specimens were gently flushed and cleaned with phosphate buffered saline (PBS, pH 7.4) and then fixed with bursa in 10% buffered formalin for subsequent morphometric analysis.

Table 2. The main active compounds of peppermint (*Mentha piperita*).*

Initial concentration	DPPH Analysis (Inhibition %)		CUPRAC Analysis (Inhibition %)	
	Water Extract	Methanol Extract	Water Extract	Methanol Extract
1000 µg/ml	81.03	85.19	3.74	3.63
500 µg/ml	64.78	74.78	2.32	2.31
250 µg/ml	47.75	56.75	1.26	1.25
125 µg/ml	34.57	42.57	0.81	0.80
62.5 µg/ml	27.85	31.85	0.54	0.54
Content ± SD	Water Extract	Methanol Extract		
Inhibition% ± SD				
Total Phenolic	102.671 ± 0.98	90.879 ± 1.02		
Total flavonoid	21.464 ± 0.36	26.023 ± 0.61		
AChE	90.28 ± 0.36	94.16 ± 0.25		
BChE	86.06 ± 1.12	88.55 ± 0.19		

DPPH (diphenyl-2-picrylhydrazil Inhibition), CUPRAC (CUPric Reducing Antioxidant Capacity), (AChE) Acetylcholinesterase, (BChE) Butyrylcholinesterase. *Own analysis.

Measurements and processing

Histology: Fixed samples of intestine and bursa of Fabricius were dried, cleared and fixed in paraffin wax for later histological analysis. The sequent longitudinal sections (7 µm) were placed individually onto Superfrost® slides (Thermo Scientific, Rockville, MD, USA) and stained with hematoxylin and eosin. Villus height, villus apical width at the tip of the villus, villus basal width at the crypt-villus junction, crypt depth, muscularis depth (thickness), length of lymphoid follicles, width of lymphoid follicles, and follicular area were measured on 10 villi and 10 lymphoid follicles of bursa per sampled broiler using the Dino-eye program by analyzing the images captured with a color video camera (Dino-eye 20). The villi height: crypt depth ratio (VH:CD) per broiler was calculated by dividing the average of the 10 measured villi heights by the average of the 10 measured crypt depth. of the same broiler. The apparent villus surface area was found out using the formula: $\{[(\text{villus tip} + \text{villus base})/2] \times \text{villus height}\}$ (21). The area of bursa lymphoid follicles was measured by drawing a around the selected follicles.

Serum biochemical

On day 24 of age, peripheral blood samples were taken from the jugular vein of two birds per pen and serum was separated for estimating of total protein, albumin, cholesterol, alanine transaminase (ALT) and

aspartate aminotransferase (AST) using an automatic COBAS INTEGRA400 plus analyzer (Cedex Bio HT Analyzer).

Statistical Analysis

The SAS statistical package (PROC GLM) was used to test the homogeneity of variances and normality of data as well as to determine significance and interactions of main effects (SAS, 2013). When interactions were observed ($P < 0.05$), Duncan's multiple range test was used to detect the differences between individual treatment means.

RESULTS AND DISCUSSION

Broilers' performance

Performance results of broilers are show in Tables 3-5. Table 3 shows that from d 0 to d 10, challenged broilers had lower weight gain (WG) ($P < 0.05$) and strongly tended to have poorer feed conversion ratio (FCR) ($P = 0.0520$) compared to non-challenged birds. Feed intake (FI) of broilers given 1% peppermint was lower than those given salinomycin ($P < 0.05$). Challenge x additive interaction was existing for FI ($P < 0.05$) but was not for WG and FCR ($P > 0.05$). Challenged broilers in 1% peppermint treatment consumed less feed ($P < 0.05$) compared to those in Negative Control and non-challenged salinomycin groups of broilers. From d 0 to d 24 there were no significant differences in growth performance of challenged and non-challenged broilers despite of *Eimeria*'s strong tendency to

negatively affect FCR ($P = 0.0566$) (Table 4). Broilers given both concentrations of peppermint and salinomycin had higher WG ($P < 0.0001$) and better FCR ($P < 0.05$) compared to controls. Broilers fed 0.5% peppermint had higher WG than those fed 1% peppermint ($P < 0.0001$). Challenge x additive interactions were present for WG and FCR ($P < 0.0001$) as well as for FI ($P < 0.05$). Positive

(challenged) control broilers had lower WG compared to those of the all other treatments and negative (non-challenged) controls ($P < 0.0001$). On the other hand, broilers of the all challenged and non-challenged treatments except the challenged ones fed 1% peppermint had higher WG compared to negative controls ($P < 0.0001$).

Table 3. Effect of different treatments on broilers' performance from d 0 to d 10 of age.

Treatments*	Weight gain g/bird	Feed Intake g/bird	FCR
Negative control	247.75	295.500 ^{ab}	1.199
NC Salinomycin	253.75	302.500 ^a	1.194
NC 0.5% peppermint	255.00	291.750 ^{bc}	1.147
NC 1% peppermint	249.50	295.000 ^{ab}	1.184
Positive control	234.25	293.000 ^{bc}	1.254
C Salinomycin	249.25	296.750 ^{ab}	1.193
C 0.5% peppermint	235.25	296.250 ^{ab}	1.263
C 1% peppermint	233.25	286.000 ^c	1.229
Pooled SEM	2.786	1.118	0.013
Main effects			
Challenge			
None	252.75 ^a	296.42	1.175
<i>Eimeria</i>	239.25 ^b	293.00	1.228
Additive			
Control	241.00	294.25 ^{ab}	1.226
Salinomycin	251.50	299.63 ^a	1.193
0.5% Peppermint	245.13	294.00 ^{ab}	1.205
1% Peppermint	241.38	290.50 ^b	1.207
P > F			
Challenge	0.0255	0.2494	0.0520
Additive	0.5344	0.0275	0.8491
Challenge x additive	0.2343	0.0107	0.3445

^{a, b} – Means within the same column with different superscripts differ significantly at ($P < 0.05$).

*Negative control = Basal diet (No additive + No challenge). Positive control = Basal diet (No additive + challenged with *Eimeria*). NC = Non-challenged with *Eimeria*. C = Challenged with *Eimeria*.

Furthermore, broilers fed 0.5% peppermint had markedly higher WG compared to those of the all other challenged and non-challenged treatments ($P < 0.0001$). Negative control broilers and non-challenged ones fed 1% peppermint consumed less feed compared to those of the all other groups except the challenged broilers fed salinomycin ($P < 0.05$). Positive control broilers had poorer FCR compared to those of the all other challenged and non-challenged treatments ($P < 0.0001$). Also, FCR of non-challenged broilers fed 0.5% peppermint was better than challenged broilers fed 1% peppermint and negative

controls ($P < 0.0001$). Table 5 demonstrates that from d 0 to d 35, challenged broilers had lower WG ($P < 0.0001$), consumed less feed and had poorer FCR ($P < 0.05$) compared to non-challenged broilers. Salinomycin, 0.5% and 1% peppermint fed broilers had improved WG and FCR but not FI compared to controls ($P < 0.05$). Challenge x additive interactions were observed for WG and FCR ($P < 0.0001$) as well as for FI ($P < 0.05$). In general, the interactions pointed out that adding of peppermint or salinomycin exerted a greater positive effect on the WG and FCR of *Eimeria* challenged broilers compared to positive

controls than in non-challenged broilers compared to negative controls. Positive control broilers had lower WG and poorer FCR compared to negative control and the all other challenged and non-challenged broilers ($P < 0.0001$). Also, non-challenged broilers fed salinomycin and both concentrations of

peppermint had greater WG compared to challenged broilers given 0.5% and 1% of peppermint ($P < 0.0001$). Non-challenged broilers in both peppermint groups consumed more feed than those in negative control and challenged ones in both peppermint and salinomycin groups ($P < 0.05$).

Table 4. Effect of different treatments on broilers' performance from d 0 to d 24 of age.

Treatments*	Weight gain g/bird	Feed Intake g/bird	FCR
Negative control	982.25 ^c	1369.00 ^b	1.394 ^b
NC Salinomycin	1057.00 ^b	1432.5 ^a	1.356 ^{bc}
NC 0.5% peppermint	1134.50 ^a	1463.75 ^a	1.291 ^c
NC 1% peppermint	1052.75 ^b	1379.5 ^b	1.313 ^{bc}
Positive control	881.75 ^d	1466.75 ^a	1.670 ^a
C Salinomycin	1053.75 ^b	1417.50 ^{ab}	1.345 ^{bc}
C 0.5% peppermint	1068.25 ^b	1437.25 ^a	1.346 ^{bc}
C 1% peppermint	1039.00 ^{bc}	1432.50 ^a	1.379 ^b
Pooled SEM	13.878	7.852	0.022
Main effects			
Challenge			
None	1081.42	1425.25	1.320
<i>Eimeria</i>	1053.67	1429.08	1.357
Additive			
Control	932.00 ^c	1417.88	1.532 ^a
Salinomycin	1055.38 ^{ab}	1425.00	1.351 ^b
0.5% Peppermint	1101.38 ^a	1450.50	1.318 ^b
1% Peppermint	1045.88 ^b	1406.00	1.346 ^b
P > F			
Challenge	0.1073	0.8055	0.0566
Additive	<.0001	0.2347	0.0002
Challenge x additive	<.0001	0.0022	<.0001

^{a, b, c} – Means within the same column with different superscripts differ significantly at ($P < 0.05$). *See Table 3 for details of treatments

Table 5. Effect of different treatments on broilers' performance from d 0 to d 35 of age.

Treatments*	Weight gain g/bird	Feed Intake g/bird	FCR
Negative control	1894.75 ^{ab}	2794.25 ^b	1.475 ^b
NC Salinomycin	1957.00 ^a	2829.25 ^{ab}	1.446 ^b
NC 0.5% peppermint	1974.25 ^a	2915.25 ^a	1.477 ^b
NC 1% peppermint	1977.75 ^a	2924.75 ^a	1.478 ^b
Positive control	1698.00 ^c	2859.00 ^{ab}	1.686 ^a
C Salinomycin	1902.75 ^{ab}	2783.75 ^b	1.463 ^b
C 0.5% peppermint	1860.50 ^b	2786.75 ^b	1.498 ^b
C 1% peppermint	1844.50 ^b	2767.00 ^b	1.500 ^b
Pooled SEM	17.408	15.650	0.014
Main effects			
Challenge			
None	1969.67 ^a	2889.75 ^a	1.467 ^b
<i>Eimeria</i>	1869.25 ^b	2779.17 ^b	1.488 ^a
Additive			
Control	1796.38 ^b	2826.63	1.58025 ^a
Salinomycin	1929.88 ^a	2806.50	1.45450 ^b
0.5% Peppermint	1917.38 ^a	2851.00	1.48725 ^b
1% Peppermint	1911.13 ^a	2845.88	1.48925 ^b
P > F			
Challenge	<.0001	0.0007	0.0386
Additive	0.0151	0.7579	0.0051
Challenge x additive	<.0001	0.0370	<.0001

^{a, b, c} – Means within the same column with different superscripts differ significantly at ($P < 0.05$). *See Table 3 for details of treatments

Meat type chickens' performance is highly related to their gut's growth and health. The gut's health has a significant role in attaining high productivity. It influences feed digestion, nutrients metabolism and their absorption and utilization particularly energy and protein as well as it affects the resistance of broilers to diseases (29). In the last few decades medicinal plants such as Peppermint, Oregano, Rosemary etc. have been interestingly used as feed additives for improvement of broilers' performance under normal conditions. However to my knowledge there are no published data on the effect of whole peppermint plant and little ones on the effect of essential oils derived from peppermint in controlling of coccidiosis in broilers. In the current study, the coccidiosis infection was successfully induced as demonstrated by declined WG and FI as well as poor FCR in challenged broilers at the all three phases of the rearing. The positive effect of peppermint on the performance of broilers was obviously appeared from d 0 to 24 and d 0 to 35 of age. Peppermint not only had the same ability of salinomycin in protecting the performance of challenged broilers, but also greatly improved the WG and FCR of challenged and non-challenged broilers. This was similar case of Barbour et. al., (8) who reported that giving coccidian-challenged broilers a commercial blend containing eucalyptus and peppermint essential oils (EOs) in drinking water had increased body weight (BW), improved FCR, and decreased mortality percentage. Also Nameghi et. al., (32) demonstrated that adding a blend of essential oils (EOB) extracted from thyme, peppermint and eucalyptus to drinking water had improved broilers WG and FCR. Similarly, Abdullah and Al-Barwary (3) found that addition of *Artemisia* as a medicinal plant to feed and drinking water of coccidiosis challenged broilers improved their WG and FCR. Furthermore, Hasan and M'Sadeq (16) demonstrated that WG and FCR of *E. coli* challenged broilers were enhanced when given diets and drinking water supplemented with peppermint powder. In contrast, Asadi et al., (7) and Ocak et al., (33) found that in-feed giving of peppermint to broilers did not

significantly improved their daily WG at starter and finisher periods of rearing. The beneficial effects of peppermint powder or its derived EOs on the performance of the broilers could be due to the improvement of feed digestibility and nutrients' absorption especially lipids. This might be resulted from the peppermint and other herbal plants' content of active compounds like carvacrol, menthol, thymol, ρ -cymene, 1,8-cineol, menthone, γ -terpinene and α -pinene which stimulate the secretion of saliva and endogenous enzymes like amylase and enhance the synthesis of bile acid in liver and the excretion of bile acids which will improve the digestion and absorption of lipids (14, 31, 40). The better growth performance of broilers in the current experiment might be also due to the significant improvement of their gut health through increasing of villus height and VH:CD ratio and reducing of crypt depth.

Visceral organs relative weight

Relative weight of broilers' visceral organs to their live body weight are illustrated in Table 6. It is noted that *Eimeria* challenged broilers had significantly lower relative weight of liver in comparison with non-challenged ones. 0.5% peppermint supplemented broilers had relatively heavier intestines weight compared to the all other treatments ($P < 0.05$). There was a tendency for the relative weight of bursa of Fabricius of broilers of both additives particularly 0.5% peppermint to be higher than those of the controls ($P = 0.099$). Also a strong tendency of challenge \times additive interaction was observed for relative weight of intestines ($P = 0.059$). In the present study the relative weight of edible visceral organs was not influenced by experimental factors except that the relative weight of liver of challenged broilers was less than those of non-challenged ones. This was in agreement with what demonstrated by Huff and Ruff (20) that *Eimeria tenella* significantly decreased the relative weight of broilers' liver compared to un-inoculated broilers. In contrast, Brito et al., (10) found that *Eimeria maxima* significantly increased the relative weight of the liver.

Table 6. Effect of different treatments on relative internal organs' weights (g/100g BW) of broilers at d 24 of age.

Treatments*	Liver	Heart	Spleen	Gizzard	Small Intestine	Bursa of Fabricius
Negative control	3.11	0.78	0.10	3.73	7.08	0.147
NC Salinomycin	3.19	0.71	0.09	3.82	6.64	0.153
NC 0.5% peppermint	3.73	0.99	0.16	4.36	10.10	0.238
NC 1% peppermint	3.20	0.74	0.09	4.57	7.20	0.167
Positive control	2.82	0.61	0.10	3.96	6.54	0.210
C Salinomycin	2.77	0.57	0.10	3.73	6.06	0.206
C 0.5% peppermint	2.81	0.62	0.11	3.87	7.68	0.236
C 1% peppermint	2.76	0.61	0.11	4.13	6.38	0.192
Pooled SEM	0.100	0.045	0.007	0.101	0.337	0.010
Main effects						
Challenge						
None	3.374 ^a	0.814	0.110	4.250	7.980	0.186
<i>Eimeria</i>	2.777 ^b	0.603	0.105	3.910	6.708	0.211
Additive						
Control	2.964	0.693	0.104	3.842	6.811 ^b	0.178
Salinomycin	2.981	0.642	0.094	3.776	6.350 ^b	0.179
0.5% Peppermint	3.268	0.808	0.133	4.117	8.889 ^a	0.237
1% Peppermint	2.977	0.676	0.096	4.347	6.793 ^b	0.180
P > F						
Challenge	0.012	0.072	0.780	0.203	0.158	0.331
Additive	0.678	0.621	0.120	0.167	0.027	0.098
Challenge x additive	0.190	0.351	0.204	0.338	0.059	0.139

^{a, b} – Means within the same column with different superscripts differ significantly at ($P < 0.05$). *See Table 3 for details of treatments.

In the current study the absence of peppermint's effectiveness on the relative weight of edible internal organs of broilers is in consistent with findings of Abdel-Wareth et al., (2) who reported that supplementation of menthol and peppermint leaves to broiler's diet did not affect the relative weight of pancreas, gizzard, heart, liver and spleen. However, Asadi et al., (7) stated that different levels of peppermint significantly increased the relative weight of broilers' gizzard, liver and heart.

Gut morphology

Eimeria challenged broilers had significantly shorter jejunal villi, deeper crypt, lower ratio of villus height to crypt depth (VH:CD) and wider villus base ($P < 0.05$, $P < 0.0001$) compared to non-challenged broilers (Table 7). Salinomycin and both peppermint concentrations fed broilers had longer jejunal villi ($P < 0.0001$) compared to those fed no additives. Also, salinomycin fed broilers had

longer villus in comparison with those fed both peppermint concentrations ($P < 0.0001$). Crypt depth was reduced in broilers given 0.5% and 1% peppermint ($P < 0.05$) compared to those fed no additives diet and salinomycin. Broilers fed basal diet without additives had wider villus tip compared to those fed salinomycin and both peppermint concentrations ($P < 0.05$). Both concentrations of peppermint significantly decreased width of broilers' jejunal villus base compared to the controls and reduced apparent surface area of villus in comparison with those fed salinomycin added and non-supplemented control diets ($P < 0.05$). The thickness of jejunum muscle of broilers fed 0.5% peppermint was lower ($P < 0.0001$) than those of control and salinomycin groups. In addition salinomycin fed broilers had thicker jejunal muscles ($P < 0.0001$) compared to those fed both peppermint concentrations. Challenge x additive interactions were markedly present

for villus height, crypt depth, VH:CD ratio, thickness of jejunum muscle at ($P < 0.0001$) and for width of villus tip, width of villus base, apparent villus surface area at ($P < 0.05$). The villus height and jejunum muscle thickness of non-challenged salinomycin fed broilers were higher than those of the all other non-challenged treatments ($P < 0.0001$). Also, non-challenged control broilers had significantly wider villus tip and base compared to those of the all other non-challenged broiler groups. Furthermore, broilers of non-challenged control had larger apparent villus surface area compared to those of non-challenged 0.5% and 1% peppermint ones ($P < 0.05$). However, there were not significant differences between treatments in terms of crypt depth and VH:CD ($P > 0.05$). Under the coccidiosis challenge condition, peppermint had positive impact on the morphology of the jejunum. Broilers received salinomycin and both peppermint concentrations showed an increase in villus

height and VH:CD ratio, and a decrease in crypt depth and thickness of jejunum muscle with respect to those fed control diet ($P < 0.0001$). In addition, salinomycin fed broilers had taller villus and thicker jejunum muscle compared to those fed only 0.5% peppermint ($P < 0.0001$), and had deeper crypt ($P < 0.0001$) and wider villus base ($P < 0.05$) compared to those fed both peppermint concentrations. Moreover, broilers given salinomycin had larger apparent villus surface area in comparison with control, 0.5% and 1% peppermint fed ones ($P < 0.05$). However, there were no significant differences in width of the villus tip between control broilers and those fed salinomycin and both peppermint concentrations. General health status and subsequent growth performance are directly correlated to their gastrointestinal health. Integrity and health of bird's alimentary tract is related to their gut morphology.

Table 7. Effect of different treatments on jejunum muscle thickness, villus height, crypt depth and apparent villus surface of broilers at d 24 of age.

Treatments*	Villus height μm VH	Crypt depth μm CD	VH/CD ratio	villus tip width μm	villus base width μm	apparent villus surface area μm^2	Jejunum muscle thickness μm
Negative control	945 ^{bc}	183 ^c	5.55 ^a	241 ^a	247 ^a	230151 ^a	204 ^c
NC Salinomycin	1109 ^a	211 ^c	5.43 ^a	163 ^b	169 ^b	185019 ^{ab}	265 ^b
NC 0.5% peppermint	997 ^b	172 ^c	5.89 ^a	137 ^b	162 ^b	148643 ^b	212 ^c
NC 1% peppermint	939 ^{bc}	171 ^c	5.65 ^a	163 ^b	168 ^b	156172 ^b	210 ^c
Positive control	762 ^d	402 ^a	1.91 ^c	179 ^b	208 ^{ab}	146084 ^b	330 ^a
C Salinomycin	1008 ^b	311 ^b	3.82 ^b	182 ^b	242 ^a	211454 ^a	262 ^b
C 0.5% peppermint	914 ^c	231 ^c	4.09 ^b	139 ^b	174 ^b	142659 ^b	208 ^c
C 1% peppermint	977 ^{bc}	229 ^c	4.39 ^b	156 ^b	164 ^b	157465 ^b	242 ^{bc}
Pooled SEM	9.59	7.42	0.13	5.74	6.89	5630.99	4.87
Main effects							
Challenge							
None	1015 ^a	186 ^b	5.61 ^a	158	167 ^b	165793	231
<i>Eimeria</i>	975 ^b	270 ^a	4.02 ^b	165	206 ^a	180422	243
Additive							
Control	889 ^c	251 ^a	4.43	222 ^a	235 ^a	204284 ^a	243 ^{ab}
Salinomycin	1064 ^a	255 ^a	4.72	172 ^b	201 ^{ab}	196650 ^a	264 ^a
0.5% Peppermint	959 ^b	199 ^b	5.06	138 ^b	168 ^b	145881 ^b	210 ^c
1% Peppermint	948 ^b	185 ^b	5.34	162 ^b	167 ^b	156487 ^b	218 ^{bc}
P > F							
Challenge	0.0469	<.0001	<.0001	0.5159	0.0060	0.2027	0.2724
Additive	<.0001	0.0002	0.1187	0.0015	0.0120	0.0004	<.0001
Challenge x additive	<.0001	<.0001	<.0001	0.0075	0.0013	0.0006	<.0001

a, b, c – Means within the same column with different superscripts differ significantly at ($P < 0.05$). *See Table 3 for details of treatments

The VH:CD ratio is a significant index of retrieval and health. High ratio indicating a long, mature, active and functional villus accompanying with a thin crypt and continual renewal of cells (30). Findings of the current study revealed that coccidian has dramatically changed the gastrointestinal morphology of broilers. At d 24 of age and post *Eimeria* challenge, positive control (challenged control) broilers had significantly shorter villus length, lower VH:CD ratio and deeper crypt compared to negative control (non-challenged control) and challenged broilers fed salinomycin and peppermint supplemented diets. This was similar to outcomes of a study by M'Sadeq et al., (30) when *Eimeria* has severely impaired the morphology of broilers' jejunum. Authors indicated that existence of pathogens in the gut is considered to enterocyte cells damage resulting in deeper crypts. The deeper crypts would require higher energy utilization for maintaining of intestine instead of its using for improvement of growth performance. This means that in the current study peppermint had the same ability of salinomycin in improvement of the health and morphology of coccidian challenged broilers. In the recent times a great attention has been paid to aromatic plants and their extracts. This is due to their capability of gut flora manipulation (17), antimicrobial characteristics by changing the properties of cell membranes and causing ion leakage and subsequent reduction of microbes virulence (35, 42), boosting of the immune response (15) as well as antiparasitic and coccidiostat properties through their flavonoid and phenolic compounds by retarding the growth and reproduction of coccidian parasite and decreasing of its oocyst count (5, 43). In the present study broilers' gut health protection and its morphological improvement resulted from peppermint supplementation are similar to findings of Mehri et al., (26) who demonstrated that in-feed peppermint increased the VH:CD ratio in ileum of quails. Similarly Hasan and M'Sadeq (16) found that peppermint in feed or drinking water of *E. coli*

challenged broilers increased the VH:CD ratio while it had no effect on villus height. Also Abdullah and Al-Barwary (3) demonstrated that *Artemisia splendens* as a herb increased villus height and VH:CD ratio and decreased crypt depth of *Eimeria* challenged broilers' jejunum. However, Guo et al., (15) reported that the medicinal plants had no significant impact on the morphometric measurements of intestine when compared with control and antibiotic.

Cloacal bursa morphology

The morphometric measurements of bursa of Fabricius at d 24 of age are given in Table 8. The bursal lymphoid follicles of *Eimeria* challenged broilers had longer, wider and larger area compared to those of non-challenged broilers ($P < 0.05$). There were no significant differences between additives fed broilers and controls in bursa morphological parameters ($P > 0.05$). Challenge x additive interactions were observed for length and area of bursa lymphoid follicles ($P < 0.05$). The challenged broilers fed control diet with no additives had significantly longer bursal lymphoid follicles compared to challenged ones fed 1% peppermint and larger area of bursal lymphoid follicles compared to challenged broilers fed 0.5% and 1% peppermint. No significant differences were observed between non-challenged treatments of broilers in terms of bursa morphometric measurements. The bursa of Fabricius as one of the primary lymphoid organs is the site of the B lymphocytes differentiation and development involves in the acquired immune function by secretion of antibodies that bind to antigens (34). The integrity of bursa and other lymphoid tissues is critical for proper immune modification (4). In the present study the numerical increase in the relative weight of bursa of Fabricius and the significant increase in the length, width, and area of its lymphoid follicles in *Eimeria* challenged broilers might be due to the enlargement of its epithelium resulted from developing of *Eimeria tenella* in the bursa of Fabricius (6, 18).

Table 8. Effect of different treatments on morphometric cloacal bursa measurements of broilers at d24 of age.

Treatments*	Bursa Lymphoid follicles length μm	Bursa Lymphoid follicles width μm	Bursa Lymphoid follicles area μm^2
Negative control	5018 ^b	2985	12909 ^b
NC Salinomycin	4559 ^b	3020	11563 ^b
NC 0.5% peppermint	4649 ^b	2847	10845 ^b
NC 1% peppermint	4774 ^b	2967	12265 ^b
Positive control	6576 ^a	3995	21532 ^a
C Salinomycin	5610 ^{ab}	3604	16538 ^{ab}
C 0.5% peppermint	5604 ^{ab}	3323	14592 ^b
C 1% peppermint	5323 ^b	3407	14024 ^b
Pooled SEM	156.6	118.2	896.7
Main effects			
Challenge			
None	4661 ^b	2945 ^b	11558 ^b
<i>Eimeria</i>	5512 ^a	3445 ^a	15051 ^a
Additive			
Control	5797	3490	17221
Salinomycin	5085	3312	14050
0.5% Peppermint	5127	3085	12718
1% Peppermint	5049	3187	13145
P > F			
Challenge	0.0050	0.0035	0.0110
Additive	0.2818	0.6728	0.2847
Challenge x additive	0.0081	0.2024	0.0484

a, b – Means within the same column with different superscripts differ significantly at ($P < 0.05$). *See Table 3 for details of treatments.

Anderson et al., (6) and Helal et al., (18) have reported the existence of *Eimeria tenella*'s life cycle in the bursa of Fabricius of broilers. Bursal fold and lymphoid follicles' epithelial hyperplasia might be an indicator of the presence of an early cellular immune function against invading *Eimeria tenella* (22). This was investigated by Ilić et al. (22) through detecting of CD3-T lymphocyte population in bursa of Fabricius of *Eimeria tenella* infected broilers. In the present study, the significantly less enlarged bursa of challenged peppermint fed broilers compared to challenged controls could be due to their better intestine's health and integrity resulted from Oocysticidal activity of peppermint's essential oils by decreasing of coccidian Oocyst count (13).

Serum biochemical profile

The effect of *Eimeria* and different additives on the broilers' serum biochemical traits at d 24 of age are presented in Table 9. The results

revealed that, challenged broilers with no additives had higher serum content of cholesterol ($P < 0.0001$), ALT, AST, and albumen ($P < 0.05$). There were not significant differences between control fed broilers and those fed salinomycin, 0.5% and 1% peppermint supplemented diets in the studied serum biochemical parameters ($P > 0.05$). Under *Eimeria* challenge conditions, the only 0.5% peppermint decreased serum content of cholesterol and ALT however both peppermint concentrations lowered AST content of serum compared to positive controls ($P < 0.05$), however, non-challenged treatments of broilers were not significantly different. The current study showed that *Eimeria* challenge significantly affected broilers' serum biochemical parameters like cholesterol, AST, ALT, and albumen. Similarly, Hirani et al., (19) found an increase in the serum level of cholesterol and serum AST and ALT activities

in coccidian challenged broilers. However Mondal et al., (27) showed that *Eimeria* challenged broilers' plasma level of ALT and albumen were significantly decreased. The observed increase in serum cholesterol concentration of coccidian challenged broiler' in the current study might be due to impairment in liver which will lead to disturbed fat metabolism resulted from damaged intestinal epithelium in coccidiosis (9). In addition the significant increase in the serum AST and ALT activity in challenged

broilers in the present experiment could be due to the injured liver and intestine (19) or it might be due to degeneration of cell membrane and tissues (28).

CONCLUSION

The current study was succeeded in performing the coccidiosis challenge model. It demonstrated that peppermint was similar to anticoccidial in controlling performance declining of broilers represented by WG and FCR. It was also as efficient as anticoccidial in improving of intestine

Table 9. Effect of different treatments on serum biochemical parameters of broilers at d 24 of age.

Treatments*	Cholesterol (mg/dl)	ALT (ul)	AST (ul)	Total Protein (g/dl)	Albumen (g/dl)	Globulin (g/dl)
Negative control	91 ^{cd}	2.43 ^{cd}	158 ^c	2.533	1.058	1.475
NC Salinomycin	86 ^d	2.30 ^{cd}	136 ^c	1.795	0.710	1.085
NC 0.5% peppermint	85 ^d	2.13 ^d	143 ^c	2.590	0.948	1.643
NC 1% peppermint	94 ^{cd}	2.90 ^{bcd}	157 ^c	2.333	0.948	1.385
Positive control	141 ^a	4.70 ^a	244 ^a	2.350	1.015	1.335
C Salinomycin	120 ^{ab}	3.80 ^{ab}	213 ^{ab}	2.573	1.065	1.508
C 0.5% peppermint	113 ^{bc}	3.28 ^{bcd}	185 ^{bc}	2.470	1.048	1.423
C 1% peppermint	124 ^{ab}	3.53 ^{abc}	182 ^{bc}	2.508	1.050	1.458
Pooled SEM	4.247	0.194	8.119	0.071	0.031	0.046
Main effects						
Challenge						
None	88 ^b	2.44 ^b	145 ^b	2.239	0.868 ^b	1.371
<i>Eimeria</i>	119 ^a	3.53 ^a	193 ^a	2.517	1.054 ^a	1.463
Additive						
Control	116	3.56	201	2.441	1.036	1.405
Salinomycin	103	3.05	174	2.184	0.888	1.296
0.5% Peppermint	99	2.70	164	2.530	0.998	1.533
1% Peppermint	109	3.21	170	2.420	0.999	1.421
P > F						
Challenge	<.0001	0.0026	0.0011	0.0919	0.0053	0.4193
Additive	0.5131	0.4823	0.3854	0.3729	0.3851	0.3678
Challenge x additive	0.0001	0.0022	0.0018	0.0900	0.0577	0.1401

a, b, c, d – Means within the same column with different superscripts differ significantly at (P < 0.05). ALT= Alanine transaminase, and AST = Aspartate aminotransferase (AST). *See Table 3 for details of treatments.

integrity by increasing villus height and VH:CD ratio and decreasing crypt depth in *Eimeria* challenged broilers. These findings suggest that peppermint can be a promising preventative alternative to anticoccidial for reducing of coccidiosis acuteness.

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