

EFFECT OF *PEPPERMINT* SUPPLEMENTATION AS POWDER OR EXTRACT ON BROILER PERFORMANCE, SERUM BIOCHEMICAL CONTENT AND GUT HEALTH UNDER *E COLI* CHALLENGE

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ABSTRACT

The aim of this study was to evaluate the efficacy of different levels of peppermint (*Mentha piperita*) on broiler performance, jejunum morphology, organs percentage and serum biochemical content. A total of 576-day old ross 308 chickens was divided randomly into 12 treatments with 4 replicates pen per treatment and 12 birds each pen. Treatments were arranged in a 2 × 6 factorial arrangement of treatments was employed. Factors were challenge, - or +; control, antibiotic, 0.5 % peppermint in feed, 1% peppermint in feed, 0.5% peppermint in water and 1% peppermint in water. The result of study was showed that the peppermint supplementation was significant effect on feed intake, feed conversion ratio and it has effect on body weight at day 24 and 35. The peppermint supplementation either in feed and water at 24 day had a significant effect on jejunum muscle thickness and villus/ crypt ratio while not effect on villus height and crypt depth. In conclusion, supplementation of peppermint has positive effect on broiler performance and gut morphology.

Key words: meat chickens, productivity, gut morphology, *Escherichia coli*, peppermint

حسن وصديق

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تأثير اضافة النعناع على الاداء الانتاجي و المحتوى الكيميائي لمصل الدم وصحة الامعاء لفروج اللحم تحت تاثير الاصابة الايكولاي

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الباحث

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المستخلص

اجريت هذه الدراسة لتقييم تأثير مستويات مختلفة من النعناع (*Mentha piperita*) على الاداء الانتاجي، الصفات النسيجية للامعاء، الوزن النسبي للاعضاء الداخلية ومحتوى الكيمياء الحيوية في مصل الدم لدجاج فروج اللحم. مجموع 576 دجاج فروج اللحم بعمر يوم واحد تم تقسيمها بشكل عشوائي على 12 معاملات مع 4 مكررات لكل معاملة و 12 طائر لكل مكرر . تم ترتيب المعاملات بشكل 2*6 تجريبية عواملية. ربيت الطيور في غرفتين مختلفتين بحيث في الغرفة الاولى تم تربية الدجاج تحت الظروف الطبيعية اما في الغرفة الثانية تعرضت الطيور الى الاصابة ببكتريا الايكولاي. وزعت المعاملات في كل غرفة بالشكل الاتي (السيطرة، مضاد حيوي، 0.5% نعناع في الأعلاف، 1% نعناع في الأعلاف، 0.5% نعناع في الماء و 1% نعناع في الماء). وأظهرت نتائج الدراسة أن مكملات النعناع كان لها تأثير كبير على تناول العلف وكفاءة التحويل الغذائي ولها ايضا تأثير على وزن الجسم في اليوم 24 و 35. اضافة مكملات النعناع سواء الى الغذاء أو الى الماء في اليوم 24 كانت لها تأثير كبير على سماكة عضلات (Jejunum) و كذلك نسبة طول الزغابات الى الخبايا بينما لا تؤثر على طول الزغابات و عمق الخبايا. استنتجت هذه التجربة ان مكملات النعناع لها تأثير إيجابي على الاداء الانتاجي والصفات النسيجية لامعاء فروج اللحم.

الكلمات المفتاحية : دجاج لحم، انتاج، مجتمع مكروبي، الايكولاي، نعناع

INTRODUCTION

Commercial poultry egg and meat production dramatically increased all over the world between 1970 and 2005, poultry production was increased faster than other animal production like beef (26). This due to efficiency of the feed conversion and lower production costs related with intensive poultry production. There are many challenges related to food safety, animal well-being, production efficiency of the animal, housing environment, management (19). They face many challenges of bacterial and viral infection. Colibacillosis and salmonella are considered major bacterial diseases in the poultry industry worldwide (11). *Escherichia coli* belongs to the enterobacteriaceae family, gram-negative bacteria, growing aerobic and anaerobic with varying size and shape (20). They mainly affect intestine of birds which cause many diverse diseases, in which *Escherichia coli* (APEC) is the main reason of colibacillosis in broiler chickens (15). It has a negative economic impact on poultry production worldwide. Antibiotics have been used in the poultry industry to fight against infection disease. However, the frequent use of antibiotics may increase the incidence of antibiotic resistance. Which leads to economic losses and treatment failure, and there are also concerns about human health about the presence of antimicrobial residues in meat (7, 9). It is important to find an effective way to replace antibiotics to control infectious diseases and reduce the spread of resistant bacteria (14). Medicinal herbs have been used as alternatives to antibiotics. Medicinal plants and their products including plant extracts or essential oils are introduced as candidates for use in broiler diets and they have beneficial effects on broiler health(6). The mechanism of action of those medical plants may be through stabilization of normal gut microflora, prevention of pathogens colonization and play important role of digestive enzymes production and activities improvement (13). Peppermint is considered therapeutic herbs in the world is member from the *Labiatae* family. It is broadly utilized in herbal medicine(21). The peppermint consists of many chemical compounds which play important role as antibacterial activities, for instance, menthol in

which main phenolic compound in oil peppermint. Some studies indicated that essential oils of peppermint inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritidis*, and *Candida albicans* (22). Thus this study aimed to determine the effect of different levels of peppermint (*Mentha piperita*) in feed and water on broiler performance (weight gain, feed intake, FCR) at 10,24,35 day and histology of jejunum (villus height, crypt depth, villi/crypt ratio, muscle thickness) and organs percentage and serum biochemical at day 24.

MATERIALS AND METHODS

The study was conducted at the animal house of animal production department, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan region of Iraq.

Animal husbandry

A total of 576-day old Ross 308 chickens were divided randomly into 12 treatments with 4 replicate pen per treatment and 12 birds each pen (wire mesh partitioned at (120*75cm) will assign in two room to prevent birds from infect *E. coli*. The rooms temperature at the first day was set on 33-34 °C and then decreased by 3 °C until the end of week. Temperature and light program were adjusted according to the Ross 308 guide (Aviagen 2012). The water and feed were provided *ad libitum* the feeding program consist three periods the first is starter from 0 to 10 day and second is grower from 10-24 day, third finisher from 24 to 35. The ingredient and composition of basal diet was showed at Table 1. The primary determinants of performance, i.e., cumulative pen weight, feed intake (FI), and feed conversion ratio (FCR) were measured at d 10, 24 and 35.

Dietary treatment

In this study the negative control (no additive and non-challenge) and negative antibiotic (non-challenge and the diet 0.33 g/kg zinc bacitracin). 0.5% peppermint in feed (5 g/kg peppermint in feed and non-challenge), 1% peppermint in feed (10g/kg peppermint in feed and non-challenge) and (0.5% peppermint in water and non-challenge) (1% peppermint in water and non-challenge). However, the positive control (there is no additive and challenge and positive antibiotic (challenge and addition 0.33 g/kg zinc bacitracin in diet). 0.5% peppermint in feed (5 g/kg peppermint in

feed and challenge), 1% peppermint in feed (10g/kg peppermint in feed and challenge) (0.5% peppermint in water and challenge), (1% peppermint in water and challenge).

Preparation of powder peppermint

Fresh peppermint plant was obtained from Duhok during July and August month in 2018. At the beginning the thick stem was removed and spread on a clean cloth under the sun for 2 days to dried up.

Challenge of *E. coli*

These bacteria were isolated in a laboratory from local farms. Incubated *E. coli* was occurring at 37 °C overnight in the MacConkey broth sterile (100 ml), Then 0.1 ml of the previous broth Subsequent incubations in Eosin methylene blue agar for accounting colony. 1000 mL of MacConkey broth was used to inoculate a colony from Eosin methylene blue agar to get the vaccine challenge. Birds were inoculated with a 1.5 ml of the suspension *E. coli* (3.8×10^8 CFU per mL) at 8 and 9 day of the bird's age.

Measurements studied traits

Jejunum morphology

The fixed samples were dried, disinfected and embedded in paraffin wax for histological analysis subsequently. Successive longitudinal sections ((7□□m)) are individually placed on Superfrost® slices (Thermo Scientific, Rockville, MD, USA) and were staining with eosin and hematoxylin. The Crypt depth and Villus height was measured by Dino-eye

software and images was taken by using a color video camera (Dino-eye 20). The height of villi and depth of crypts and thickness of muscles from each replication was measured ten times. The means of crypt depth, villus height was obtaining and also villus height per crypt depth ratio was determined.

Relative organ percentage

From each pen 1 birds was picked for eviscerating to calculate the heart, spleen bursa, liver and gizzard weight at day 24.

Blood biochemical

Blood samples were collected from bird at day 24 old. Two birds from each cage were randomly selected and slaughtered. The blood was collected from jugular vein, centrifuged at 3000 rpm for 15 min and serum were taken, frozen at -20E°C. Determination of plasma total protein (TP), albumin, cholesterol, glucose, creatinine (CR), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), triglyceride (TG) and globulin were determined by automatic analyses Biolis (Tokyo Boeki Medical system) 24i by using respective biochemical kits of Cormay company the Cat. No 4-204 and 4-404 and the application of the procedure are available on (prestige 24i Biolis).

Statistical analysis

The comparison between means were carried out according to Duncan's multiple range test ($P < 0.05$) using a computerized program of SAS (SAS, 2013).

Table 1. Composition of the basal starter, grower and finisher diets as percentage

Ingredients	Starter	Grower	Finisher
Corn	47	49.9	51.5
Wheat	5	5	5
Wheat bran	5	3	5
Soybean meal	36.45	34	30
Vegetable oil	1.5	3.4	4.45
Limestone	1.8	1.69	1
Dicalcium	0.7	0.5	0.5
Salt	0.05	0.01	0.05
Vitamin premix	2.5	2.5	2.5
Nutrient composition%			
ME(kcal/kg)	2878	3035	3116
Crude protein	22.86	21.33	19.58
Crude fiber	3.02	2.76	2.92
Fat	3.76	5.64	6.8
Linoleic acid	1.92	2.88	3.48
Lysine	1.58	1.47	1.37
Methionine	0.66	0.64	0.63
Tryptophan	0.37	0.36	0.34
Methionine +cysteine	1.05	1.01	0.96
Threonine	0.97	0.92	0.86
Arginine	1.59	1.5	1.37
Calcium	1.08	0.99	0.73
Phosphor	0.54	0.5	0.49
Sodium	0.19	0.18	0.19
Chloride	0.26	0.24	0.26

RESULTS AND DISCUSSION

Broiler performance

Performance results are presented in table 2, 3 and 4. Broiler performance including body weight gain, feed intake and feed conversion ratio were measured at day 10, 24 and 35. The results of this study showed a significant effect of additives on broiler performance (Table 2). From 0-10 day the inclusion of antibiotic significantly increased weight gain and feed intake over all treatments. However, the birds fed 1% peppermint had lower feed intake compared to the control and antibiotic ($P < 0.002$). Moreover, birds fed peppermint either in water or feed decreased feed intake when compared to birds fed antibiotic. A significant challenge \times additive interaction was observed in feed intake ($p < 0.05$). The non-challenge 1% peppermint in feed treatment significantly had decrease feed intake when compared with challenged control and antibiotic. From 0-24 day the effect of additives was visible (Table 3). Birds fed Antibiotic had higher ($P < 0.01$) body weight gain over other additives. All additives had better ($P < 0.001$) feed conversion ratio when compared to control. Significant challenge \times additive interactions ($P < 0.01$) were observed in body weight gain and feed conversion. the non-challenged birds fed antibiotic significantly increase body weight gain over challenged control and non-challenged 0.5% peppermint in water. On the other hand, the challenged or no challenged birds fed peppermint either in water or feed (except non-challenged 0.5% peppermint in water) were not different in body weight gain in compare to non-challenged birds fed antibiotic. In term of feed conversion ratio, the challenged control had poorer ($P < 0.001$) feed

conversion ratio over all treatment. The non-challenged antibiotic, non-challenged 1% Peppermint in feed, challenged 0.5% and 1% Peppermint in water significantly improved feed conversion ratio in compare to non-challenged control. The performance results from day 0-35 are present in table 4. There was no significant effect of challenge on body weight gain, feed intake and feed conversion ratio. The bird's performance was markedly different upon the feed additive treatments. The highest ($P < 0.001$) body weight gain and feed intake was recorded for birds fed antibiotics. No significant different were found between birds fed control and peppermint either in water or feed for body weight gain. Birds fed control diet had significantly higher feed intake than birds fed 0.5 and 0.1 peppermint in water. All additives, except 1% peppermint in water, significantly improved feed conversion ratio over control. Challenge \times additive interactions ($P < 0.001$) was observed for body weight gain, feed intake and feed conversion ratio. Body weight gain of challenged antibiotic birds were significantly higher overall treatments except non-challenged antibiotic. Challenged birds fed control diet had lower body weight gain when compared to non-challenge 1% peppermint in feed, no challenged control and challenged control. Challenged birds fed antibiotic had significantly higher feed intake in compare to all treatments, whereas challenged birds fed 1% peppermint in water had lower feed intake, except 0.5% peppermint in water, compared to other treatments. Poorer feed conversion ratio was recorded for challenged birds fed control diet. All treatments (challenged and non-challenged) significantly improved feed conversion ratio over challenged control.

Table 2. Effect the all treatments on bird performance at day 10

Treatment	Initial weight g/birds	weight gain g/bird	Feed intake g/bird	FCR
Non challenge control	40	212	242 ^{bc}	1.143
Non challenge antibiotic	40	241	247 ^{bc}	1.028
Non challenge 0.5% Peppermint in feed	40	223	238 ^{bc}	1.071
Non challenge 1% Peppermint in feed	40	217	224 ^c	1.030
Non challenge 0.5%Peppermint in water	40	212	243 ^{bc}	1.155
Non challenge 1% Peppermint in water	40	211	236 ^{bc}	1.124
Challenge control	40	218	250 ^b	1.153
Challenge antibiotic	40	225	275 ^a	1.223
Challenge 0.5% Peppermint in feed	40	210	234 ^{bc}	1.121
Challenge 1% Peppermint in feed	40	208	228 ^{bc}	1.108
Challenge 0.5% Peppermint in water	40	221	225 ^{bc}	1.016
Challenge 1% Peppermint in water	40	211	236 ^{bc}	1.118
SEM		2.1675	2.7209	0.0158
Main effects				
Challenge				
No	40	221	238	1.081
Yes	40	215	239	1.117
Additive				
Control	40	215 ^b	246 ^{ab}	1.148
Antibiotic	40	233 ^a	261 ^a	1.125
0.5% Peppermint in feed	40	217 ^b	236 ^{bc}	1.096
1% Peppermint in feed	40	213 ^b	226 ^c	1.069
0.5% Peppermint in water	40	217 ^b	234 ^{bc}	1.085
1% Peppermint in water	40	211 ^b	236 ^{bc}	1.121
P-Value				
Challenge		0.2325	0.7777	0.3319
Additive		0.0430	0.0020	0.7436
Challenge * additive		0.0964	0.0038	0.2300

^{a-c} Means the different column was superscript are significantly different (p<0.05)

Abbreviations: SEM, standard error of the mean., FCR, feed conversation ratio

Table 3. Effect the all treatments on bird performance at day 24

Treatment	Weight gain g/bird	Feed intake g/bird	FCR
Non challenge control	1033 ^{ab}	1401 ^{ab}	1.358 ^b
Non challenge antibiotic	1122 ^a	1384 ^{ab}	1.234 ^{cde}
Non challenge 0.5% Peppermint in feed	1031 ^{ab}	1332 ^{abc}	1.292 ^{bcde}
Non challenge 1% Peppermint in feed	1061 ^{ab}	1275 ^{abc}	1.202 ^{de}
Non challenge 0.5%Peppermint in water	994 ^b	1336 ^{abc}	1.344 ^{bc}
Non challenge 1% Peppermint in water	1074 ^{ab}	1405 ^a	1.310 ^{bcd}
Challenge control	886 ^c	1352 ^{abc}	1.538 ^a
Challenge antibiotic	1083 ^{ab}	1304 ^{abc}	1.204 ^{de}
Challenge 0.5% Peppermint in feed	1047 ^{ab}	1349 ^{abc}	1.289 ^{bcde}
Challenge 1% Peppermint in feed	1019 ^{ab}	1306 ^{abc}	1.285 ^{bcde}
Challenge 0.5% Peppermint in water	1045 ^{ab}	1237 ^c	1.187 ^c
Challenge 1% Peppermint in water	1041 ^{ab}	1268 ^{bc}	1.219 ^{de}
SEM	11.9977	12.593	0.0161
Main effects			
Challenge			
No	1056	1346	1.276
Yes	1047	1293	1.237
Additive			
Control	960 ^b	1376	1.448 ^a
Antibiotic	1103 ^a	1344	1.219 ^b
0.5% Peppermint in feed	1039 ^{ab}	1340	1.290 ^b
1% Peppermint in feed	1040 ^{ab}	1290	1.244 ^b
0.5% Peppermint in water	1019 ^{ab}	1286	1.265 ^b
1% Peppermint in water	1057 ^a	1337	1.264 ^b
P-Value			
Challenge	0.6652	0.0567	0.076
Additive	0.017	0.2815	<.0001
Challenge * additive	0.0128	0.1007	<.0001

^{a-b} Means the different column was superscript are significantly different (p<0.05)

Abbreviations: SEM, standard error of the mean., FCR, feed conversation ratio

Table 4. Effect the all treatments on bird performance at day 35

Treatment	weight gain	Feed intake	FCR
	g/bird	g/ birds	
Non challenge control	1910 ^{bc}	2892 ^b	1.514 ^b
Non challenge antibiotic	2015 ^{ab}	2871 ^{bc}	1.427 ^b
Non challenge 0.5% Peppermint in feed	1921 ^{bc}	2874 ^{bc}	1.529 ^b
Non challenge 1% Peppermint in feed	1959 ^b	2826 ^{bc}	1.476 ^b
Non challenge 0.5% Peppermint in water	1856 ^{bc}	2704 ^{bc}	1.490 ^b
Non challenge 1% Peppermint in water	1914 ^{bc}	2872 ^{bc}	1.531 ^b
Challenge control	1755 ^{cd}	2861 ^{bc}	1.637 ^a
Challenge antibiotic	2159 ^a	3261 ^a	1.511 ^b
Challenge 0.5% Peppermint in feed	1993 ^b	2815 ^{bc}	1.444 ^b
Challenge 1% Peppermint in feed	1896 ^{bc}	2690 ^{bc}	1.450 ^b
Challenge 0.5% Peppermint in water	1867 ^{bc}	2652 ^{cd}	1.454 ^b
Challenge 1% Peppermint in water	1678 ^d	2474 ^d	1.512 ^b
SEM	21.9956	31.5643	0.0113
Main effects			
Challenge			
No	1933	2829	1.4906
Yes	1918	2778	1.4742
Additive			
Control	1833 ^{bc}	2876 ^b	1.575 ^a
Antibiotic	2087 ^a	3066 ^a	1.469 ^b
0.5% Peppermint in feed	1957 ^b	2845 ^{bc}	1.487 ^b
1% Peppermint in feed	1927 ^{bc}	2758 ^{bc}	1.463 ^b
0.5% Peppermint in water	1861 ^{bc}	2678 ^c	1.472 ^b
1% Peppermint in water	1796 ^c	2673 ^c	1.521 ^{ab}
P-Value			
Challenge	0.7565	0.4832	0.4392
Additive	0.0004	0.0006	0.0218
Challenge * additive	<.0001	<.0001	0.0056

^{a-c} Means the different column was superscript are significantly different ($p < 0.05$)

Abbreviations: SEM, standard error of the mean., FCR, feed conversation ratio

Jejunum morphology

The results of jejunal villus height, crypt depth, villi/crypt ratio and muscle thickness at day 24 are present in table 5. Additives had a significant effect on jejunum muscle thickness and villus/ crypt ratio. The birds fed 0.5% and 0.1% peppermint either in water or feed significantly reduced the muscle thickness compared to birds fed control diet. All additives had higher ($P < 0.01$) villus/ crypt ratio over control. There were significant challenge*additive observed in crypt depth and villus/ crypt ratio. The inclusion of additive is non-significant effect on villus height, crypt depth, muscle thickness) at the 24 day only the significant effect appears on villi/crypt ratio $p < 0.05$ in which the different

level of peppermint had significant effect on villi crypt ratio that is increase villi crypt ratio compared with control. Also, there is no significant effect between challenge and non-challenge birds $p > 0.05$ and there is non-significant challenge \times additive interactions observed $p < 0.05$ in villus height, crypt depth, villi/crypt ratio, muscle thickness.

Organs percentage

Organ percentage from live body weight results are presented in table 6. The inclusion of all peppermint additives was not affect organ percentage but only the antibiotic has decrease intestine percentage. Also there is no significant effect found between challenge and non-challenge bird and also there is no

significant \times additive interaction was observed in percentage of internal organ.

Serum biochemical

Serum biochemical results are present in Table 7 refer the effect of antibiotic, different levels of peppermint (*Mentha piperita*) in feed or water on serum biochemical (glucose, creatinine, total protein, albumin, cholesterol, triglyceride, GOT(ASAT), ALAT, globulin). At day 24 the effect of challenge was clearly visibly. Challenged birds have higher ($P<0.001$) glucose, creatinine and albumin than non-challenged birds. However, non-challenged birds had significantly higher total protein and globulin than challenged birds. No significant effect of additives was observed in glucose, creatinine, total protein, albumin, cholesterol, triglyceride, (ASAT), ALAT, globulin. There were challenge*additive interaction observed for glucose, total protein,

albumin and globulin. Challenged control birds had highest serum glucose concentration compare to non-challenged treatments. While the birds fed challenge 1% peppermint in water had significantly lower serum glucose compared to challenged treatments. The serum total protein concentration of challenged birds fed 1% peppermint in feed or water and 0.5 % peppermint in water were significantly lower than birds fed challenged antibiotic and non-challenged 0.5 peppermint in water. The serum albumin concentration of challenged birds fed antibiotic and control was higher ($P<0.001$) than all non-challenged treatments and challenged 1% peppermint in feed or water. The peppermint treatment in water and feed and antibiotic under non-challenge condition and negative control had significantly increased serum globulin compared with this treatment under *E. Coli* challenge.

Table 5. Effect of all treatments on villi height, crypt depth, muscle thickness, villi/crypt ratio at 24 day

Treatment means	Villi height um	Crypt depth Um	Muscle thickness Um	Villi / Crypt
Non challenge control	1194	224 ^{ab}	209	5.449 ^{ab}
Non challenge antibiotic	1155	157 ^c	187	7.472 ^a
Non challenge 0.5% Peppermint in feed	1693	239 ^{ab}	191	7.093 ^a
Non challenge 1% Peppermint in feed	1339	201 ^{bc}	200	6.723 ^a
Non challenge 0.5% Peppermint in water	1130	184 ^{bc}	179	6.187 ^a
Non challenge 1% Peppermint in water	1175	197 ^{bc}	174	6.055 ^a
Challenge control	1052	268 ^a	237	4.038 ^b
Challenge antibiotic	1316	241 ^{ab}	216	5.532 ^{ab}
Challenge 0.5% Peppermint in feed	1335	199 ^{bc}	185	6.826 ^a
Challenge 1% Peppermint in feed	1356	202 ^{bc}	205	6.683 ^a
Challenge 0.5% Peppermint in water	1259	215 ^{abc}	205	5.871 ^{ab}
Challenge 1% Peppermint in water	1378	218 ^{ab}	176	6.482 ^a
SEM	38.4788	6.1522	4.5253	0.2015
Main effect				
Challenge				
No	1277	193	186	6.686
Yes	1329	215	197	6.279
Additive				
Control	1123	246	223 ^a	4.744 ^b
Antibiotic	1235	199	201 ^{ab}	6.502 ^a
0.5% Peppermint in feed	1488	216	187 ^b	6.940 ^a
1% Peppermint in feed	1347	202	202 ^{ab}	6.703 ^a
0.5% Peppermint in water	1194	199	192 ^{ab}	6.029 ^a
1% Peppermint in water	1277	208	175 ^b	6.268 ^a
p-value				
Challenge	0.5667	0.0606	0.1818	0.314
Additive	0.1103	0.2015	0.0442	0.0197
Challenge*additive	0.1665	0.0172	0.1277	0.0367

^{a-b} Means the different column was superscript are significantly different ($p<0.05$)

Abbreviations: SEM, standard error of the mean

Table 6. Effect of all treatments on organ percentage at day 24

Treatment means	Intestine %	Liver %	Gizzard %	Pancreas %	Spleen %	Bursa %
Non challenge control	6.536	2.534	3.422	0.359	0.073	0.202
Non challenge antibiotic	6.048	3.313	3.788	0.326	0.097	0.273
Non challenge 0.5% Peppermint in feed	7.041	2.628	3.53	0.352	0.103	0.201
Non challenge 1% peppermint in feed	6.469	2.512	3.825	0.344	0.097	0.256
Non challenge 0.5%peppermint in water	6.389	3.138	3.691	0.356	0.112	0.24
Non challenge 1%peppermint in water	7.282	2.811	3.728	0.375	0.093	0.238
Challenge control	6.669	2.781	3.774	0.345	0.088	0.198
Challenge antibiotic	4.961	3.569	3.896	0.288	0.124	0.208
Challenge 0.5% Peppermint in feed	6.96	3.014	3.845	0.336	0.077	0.258
Challenge 1% Peppermint in feed	7.245	2.995	3.562	0.382	0.086	0.243
Challenge 0.5%Peppermint water	6.927	2.964	3.843	0.385	0.095	0.229
Challenge 1%Peppermint in water	6.954	2.931	3.45	0.385	0.095	0.227
SEM	0.157	0.0795	0.0694	0.0071	0.0039	0.0073
Main effect						
Challenge						
No	6.625	2.88	3.712	0.351	0.1	0.242
Yes	6.609	3.094	3.719	0.355	0.095	0.233
Additive						
Control	6.603 ^a	2.658	3.598	0.352 ^{ab}	0.08	0.2
Antibiotic	5.504 ^b	3.441	3.842	0.307 ^b	0.111	0.24
0.5% Peppermint in feed	6.994 ^a	2.821	3.687	0.344 ^{ab}	0.09	0.229
1% Peppermint in feed	6.857 ^a	2.753	3.694	0.363 ^a	0.091	0.25
0.5% Peppermint in water	6.658 ^a	3.051	3.767	0.371 ^a	0.103	0.234
1% Peppermint in water	7.118 ^a	2.871	3.589	0.380 ^a	0.094	0.232
p-value						
Challenge	0.9621	0.2311	0.966	0.7767	0.5447	0.5873
Additive	0.0313	0.053	0.9071	0.042	0.2654	0.5013
Challenge*additive	0.1437	0.2149	0.9454	0.1919	0.3625	0.5096

Abbreviations: SEM, standard error of the mean

Table 7. Effect all treatments on serum biochemical 24 day

Treatment means	Glu mg/dl	Cre g/dl	Tp g/dl	Alb g/dl	Cho mg/dl	Tri Mg/dl	Got U/L	ALAT U/L	Glo g/dl
Non challenge control	239 ^{dc}	0.163	3.040 ^{ab}	1.153 ^c	116	72	591	3.55	1.888 ^{ab}
Non challenge antibiotic	233 ^{dc}	0.063	3.113 ^{ab}	1.265 ^{bc}	135	103	210	4.4	1.848 ^{abc}
Non challenge 0.5% P.P in feed	241 ^{dc}	0.153	2.825 ^{abc}	1.138 ^c	89	58	234	3.3	1.688 ^{bcd}
Non challenge 1% P.P in feed	234 ^{dc}	0.078	3.185 ^{ab}	1.285 ^{bc}	117	57	281	4.05	1.900 ^{ab}
Non challenge 0.5% P.P in water	218 ^d	0.143	3.470 ^a	1.398 ^{bc}	119	71	356	4.475	2.073 ^a
Non challenge 1% P.P in water	228 ^{dc}	0.108	3.165 ^{ab}	1.305 ^{bc}	138	106	235	4.25	1.860 ^{abc}
Challenge control	335 ^a	0.198	3.285 ^{ab}	1.850 ^a	170	79	233	6.8	1.435 ^{def}
Challenge antibiotic	300 ^{ab}	0.183	3.370 ^a	1.863 ^a	150	89	268	6	1.508 ^{cde}
Challenge 0.5% P.P in feed	312 ^{ab}	0.153	2.850 ^{abc}	1.613 ^{ab}	144	89	260	4.2	1.238 ^{efg}
Challenge 1% P.P in feed	303 ^{ab}	0.18	2.635 ^{bc}	1.368 ^{bc}	144	106	252	6.275	1.268 ^{efg}
Challenge 0.5% P.P in water	298 ^{ab}	0.148	2.663 ^{bc}	1.540 ^{ab}	117	70	220	7.825	1.123 ^{fg}
Challenge 1%P.P in water	272 ^{bc}	0.145	2.243 ^c	1.270 ^{bc}	110	63	238	4.225	0.973 ^g
SEM	6.7947	0.0109	0.0703	0.0436	4.9384	5.2993	27.156	0.4098	0.0581
Main effect									
Challenge									
No	231 ^b	0.109 ^b	3.152 ^a	1.278 ^b	119	79	263	4.095	1.874 ^a
Yes	297 ^a	0.162 ^a	2.752 ^b	1.531 ^a	133	83	247	5.705	1.222 ^b
Additive									
Control	287	0.18	3.163	1.501	143	75	412	5.175	1.661
antibiotic	266	0.123	3.241	1.564	142	96	239	5.2	1.678
0.5% P.P in feed	276	0.153	2.838	1.375	117	74	247	3.75	1.463
1% P.P in feed	268	0.129	2.91	1.326	130	82	266	5.163	1.584
0.5% P.P in water	258	0.145	3.066	1.469	118	70	288	6.15	1.598
1% P.P in water	250	0.126	2.704	1.288	124	85	236	4.238	1.416
p-value									
Challenge	<.0001	0.0247	0.0088	0.0012	0.1201	0.7293	0.5378	0.07	<.0001
Additive	0.7248	0.6715	0.2129	0.4199	0.4889	0.7873	0.4247	0.6412	0.7562
Challenge*additive	<.0001	0.2917	0.0037	<.0001	0.0612	0.5144	0.2466	0.4666	<.0001

Abbreviations: SEM, standard error of the mean., (p.p) peppermint (Glu)Glucose, (Cer)creatinine, (TP)total protein, (Alb)albumin, (Cho)cholesterol, (Tri) triglyceride, (ALAT) alanine aminotransferase, (ASAT) aspartate aminotransferase, (Glo)globulin

The peppermint supplementation in feed decreased feed intake at 10 days. The results were in agreement with the finding of Aroche (5) who reported that the supplementation of mixed leaves powder of medicinal plant reduce feed intake. Also Amasaib (4) who showed that the addition of different levels of spearmint (*Mentha spicata*) improved feed intake. While, the results were in contrast with finding of Ocaik (18) who reported that the dietary supplementation of dried peppermint (*Mentha piperita L.*) and thyme

(*Thymus vulgaris L.*) leaves don't have any considerable effect on feed intake. At the 24 day the peppermint supplementation either in feed and water had improve feed conversion ratio. Also, the all peppermint additive except 1% in water had significantly improved feed conversion ratio at day 35. This result is similar to Gurbuz (10) who found that the peppermint supplementation had a significantly affect on FCR compared with control. Also Ahmed (2) they reported the addition 250 mg peppermint extract and

supplement 1 g flavomycin at the grower and Finisher periods had decrease the feed conversion ratio compared with other treatment. Also the results were agreed with Mustafa (17) study who reported that (1% betony+ 1% peppermint) had significant effect on feed conversion ratio in which have better when compared with control at age 25- 42 day. In contrast, Witkowska (27) who reported the essential oil mist in peppermint no effect on feed conversion ratio from (1 to 42 days). Most of medical plants consist of different essential oil in which stimulate digestive enzyme secretion and the effect on microbial that present in gut through the balance of microbial (6). The peppermint supplementation either in water or feed have effect on weight gain on day 24 and 35. The result was agreement with finding Witkowska (27) study who reported the addition peppermint oil mist had increase the mean BW and WG than control and birds exposed to thyme oil mist. While the result was in disagreement with the finding of Ocak (18) who recorded the addition peppermint had not any effect on body weight at 42 of age. The reason the peppermint improve bird performance may be due to that the herbs like peppermint contain different his was variety of active components which affect process of digestion in which stimulate saliva secretion and enhance bile acid synthesis in liver and bile excretion which affect process digestion and lipid absorption (8). The peppermint supplementation either in feed or water at 24 day had a significant effect on jejunum muscle thickness and villus/ crypt ratio while not affect on villus height and crypt depth. The result were agreement with Yang (28) whom reported that the addition of essential oils and organic acids in bird diet during finisher period were increased villus height to crypt depth ratio when compared to the bird fed diet with 0.15 g per kg enramycin at grower period. While, the result disagree with finding of Ahmed (2) whom recorded that the extract of peppermint had desirable effect on crypt depth and villus height in ileal in small intestine. The increase of villus height: crypt depth ratio can indicate an increase in the turnover of epithelial cells in the intestinal part due to the increase of beneficial bacteria in

mint groups(16). According to this study, the all peppermint additive in water or feed and antibiotic don't observed any considerable effect on internal organ percentage (intestine, liver, gizzard, pancreas, spleen) at day 24. The results were similar to the finding of Toghiani (24) who reported that the internal organ weight are not affect by dietary addition of peppermint . Also Ocak (18) who reported the dried peppermint (*Mentha piperita L.*) or thyme (*thymus vulgaris L.*) in diet supplements was not have any significant effect on the weights of edible organs , pancreas and gizzard. Abdel-Wareth (1) found the addition of menthol and leaves peppermint had no effect on relative weight of pancreas ,gizzard, heart ,liver, spleen .While the result was in contrast with the finding Mustafa (17) who showed the supplementation of dried peppermint 2% in broiler diet had significant increase liver and heart and gizzard percentage. According to this study, peppermint supplementation either in water or feed and antibiotic had not considerable effect on serum biochemical at day 24. The results were in agreement with finding of Khursheed (12) who reported that the supplementation of mint leaves with or without enzyme in both 1 or 2% levels were not observed any significant effect on serum glucose, total protein, cholesterol, SGPT and SGOT when compared with control. Also the results in this study were similar to the finding of Toghiani (24) who showed that the addition of different levels of black seed (*Nigellasativa*) and peppermint (*Mentha piperita*) had no significant effect on triglyceride , protein, albumin, total cholesterol. In contrast, Ahmed (2) indicated that peppermint had significant effect in which they increase the cholesterol and total protein ,globulin and liver enzyme. According the study of Akbari (3) supplementation of essential oil of peppermint and chromium picolinate under heat stress decrease the concentration of triglycerides and glucose. Also Tayeb (23) in their study found the medical plants had significant affect on albumin, globulin, cholesterol and glucose concentration. The result of serum biochemical, in this study were varied there are more factors affecting on serum biochemical. It can be nutritional situation and

compounds of herbs and also there are many other factor, like age, sex, type of bird, and environmental (25).

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