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Research Article

Effect of Peppermint Extracts Inclusion in Broiler Chick Diet on Chick Performance, Plasma Constituents, Carcass Traits and Some Microbial Populations, Enzymatic Activity and Histological Aspects of Small Intestine

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Abstract

Background: Considerable attention has been directed to including medicinal herbs and those extracts as replacers for antibiotic growth promoters in broiler diets due to the cross and multiple resistance effect of antibiotic preparations on different microbial population in small intestine and hence negatively affect the health of poultry-product consumers. **Methodology:** One hundred and eighty broiler chicks were used to evaluate the effect of dried Peppermint (*Mentha piperita*) leaves and oil inclusion in broiler diets on chick performance, carcass traits and some microbial, enzymatic and histological measurements of small intestine. Chicks fed on 6 experimental diets (starter and grower) represents; basal diet that was taken as control treatment, then supplemented per kg with 1.5 g Peppermint Leaves (PL1.5), 3.0 g Peppermint Leaves (PL3.0), 125 mg Peppermint Oil (PO125), 250 mg Peppermint Oil (PO250) or 1 g flavomycin (AGP) as a commercial antibiotic preparation. **Results:** Results of concerned study revealed the followings; feed conversion rate significantly improved with control and PO125 groups during starter period and with PO250 and AGP groups during grower and overall periods. Carcass traits didn't differ between treatments, except gizzard percent that increased with all peppermint extracts. AGP and all Peppermint treatments positively increased plasma total protein, total cholesterol, globulin and affect liver enzymes but with unclear trend. All PO and AGP treatments slightly improved the immunity indicator of chick plasma. All peppermint extracts tended to increase counts of lactobacilli and decrease common pathogenic bacteria of small intestine and enhanced the activity of stomach protease and ileal amylase and protease. Tested peppermint extracts showed a desirable effect on ileal villus height and crypt depth. **Conclusion:** The PO250 diet exhibited the best desirable results for most tested parameters compared with tested antibiotic preparation and the other experimental treatments.

Key words: *Mentha piperita*, flavomycin, performance, bacteriology, histology, plasma, enzymes, broilers

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There are many commercial antibiotic preparations uses in poultry industry in large scale to face pathogenic microorganisms as well as growth promoters. Recently, it has been documented that the use of antibiotics (AGP) in chicken diets or drinking water has caused some undesirable results for chickens and those consumers[1]. Therefore, most antibacterial performance promoters have been banned due not only to cross- resistance but also to multiple resistances[2], poultry nutritionists interested to looking for natural and safely AGP alternatives. Considerable attention has been directed to medicinal herbs as replacer for AGP[3]. Herbs, spices or their products including plant extracts, essential oils or the main components of the essential oils are among the alternative growth promoters that are already being used in research publication field[4].

Peppermint (*Mentha piperita*) is a medicinally important plant that belongs to the family Labiate[5]. The later includes many famous essential oil plants such as spearmint, basil, lavender, rosemary, sage, marjoram and thyme. The chemical analysis of peppermint leaves according to Dew *et al*.[6] indicated that those contains about 0.5-4.0% volatile oil that composed of 50-78% free menthol, thymol, monoterpene, menthofurane and traces of jasmine oil (0.15%). Even though, there are many *in-vitro* and *in-vivo* publications those stated that peppermint leaves and their essential oil and menthol have many desirable effects, including; moderate antibacterial effect on pathogenic bacteria[7], antiviral and fungicidal activity[8,9], appetizing, digestion-stimulating and antimicrobial properties[10] but there is little study information interested to whether the peppermint inclusion as a solid herb material or as essential oil form would have growth promoting effects on broiler chickens especially against commercial antibiotic from performance and intestinal histology and its pathogenic and beneficial bacteria aspects. Accordingly, concerned study was designed to investigate the effect of dietary supplementation of broiler chicks with two forms of peppermint extracts (leaves and oil) as growth promoter using some parameters represents, performance, carcass traits, essential plasma constituents and enzymatic activities, besides, the bacteria counts and histological changes of gastrointestinal tract against a commercial antibiotic preparation and the control diet.

MATERIALS AND METHODS

Birds management: A total number of 180 unsexed day-old Arbor Acres broiler chicks were fed on commercial starter diet (23% CP and 3100 kcal ME/kg) during the 1st week of age to minimize the maternal effects, then, birds were randomly distributed into 6 starter dietary treatments from 8-21 day of age and into 6 grower experimental diets from 22-35 day of age. Starting from 7 days old, chicks were reared on 12 floor pens and randomly allocated to 6 treatments of 30 chicks each in two replicates (15 chicks per replicate) until the end of experiment at 35 days old. Feed and drinking water were offered *ad libitum* for chicks those subjected daily to 22 h light and 2 h darkness along the experimental period. Periodical vaccinations of chicks were done according to the Strain Instruction Guide.

Tested dietary growth additives and experimental diets: Peppermint (*Mentha piperita*) leaves (PL) were collected, washed thoroughly in tap water and dried at dark room temperature for 15 days, then it grinded by grinder machine without any special treatments. Peppermint Oil (PO) was extracted from the whole plant above ground just before flowering. The oil was extracted by steam distillation from the fresh or partly dried plant where the yield is 0.1-1.0%. The basal diets were formulated to meet standard requirements of nutrients for broiler chicks according to NRC[11]. Composition and calculated analysis of starter and grower basal diets are described in Table 1. The basal diets were taken as the control treatment (control), while, peppermint leave powder (PL) was added to basal diets at levels of 1.5 and 3 g kg^{-1} diet

Table 1: Composition and calculated analysis of basal diets

	Basal diets	
Ingredients	Starter	Grower
Yellow corn	46.58	54.57
Soybean meal 44%	36.20	30.15
Full fat soybean	9.00	9.00
Soybean and sunflower oil	3.65	2.00
Ca carbonate	1.60	1.48
Mono calcium phosphate	1.85	1.68
HCl lysine	0.08	0.22
DL methionine	0.34	0.20
Salt (NaCl)	0.40	0.40
Broiler premix*	0.30	0.30
Total	100	100
Calculated chemical analysis		
CP (%)	23.12	21.13
ME kcal kg^{-1} diet	3071	3045
Ca (%)	1.02	0.93
Available phosphorus (%)	0.50	0.46
Lysine (%)	1.39	1.39
Methionine+cystine (%)	1.06	0.88

*Each 3 kilogram of the premix contains the followings: 12000000 IU Vit. A, 2000000 IU Vit. D3, 10000 mg Vit. E, 2000 mg Vit. K3, 1000 mg Vit. B1, 5000 mg Vit. B2, 1500 mg Vit. B6, 10 mg Vit. B12, 10000 mg Ca D-pantothenate, 30000 mg Niacin, 1000 mg Folic acid, 50 mg Biotin, 250000 mg Choline chloride, 60000 mg Mn, 50000 mg Zn, 30000 mg Iron, 10000 mg Cu, 1000 mg Iodine and 100 mg Se, where $CaCO_3$ taken as a carrier

and expressed as PL1.5 and PL3.0 treatments, respectively. By the same way the PO was added to basal diets at levels of 125 and 250 mg kg^{-1} diet and expressed as PO125 and PO250 treatments, respectively. The sixth dietary treatment in terms of AGP treatment was contained flapol flavophospholipol® (flavomycin) as growth promoter antibiotic at recommended level of 1 g kg^{-1} basal diets.

Experimental measurements

Chick performance: Feed intake and body weight were weekly recorded and corrected to mortality rate for all experimental group chicks. Then Body Weight Gain (BWG) g^{-1} chick, Feed Intake (FI) g^{-1} chick and Feed Conversion Rate (FCR) g FI g^{-1} BWG were mathematically calculated for each period and overall period.

Blood sampling and measurement of carcass traits and length of small intestine: At day 35, six birds per treatment were randomly selected and slaughtered. Blood samples were taken from their jugular vein, centrifuged at 3000 rpm for 15 min and sera were taken, frozen at -20°C until biochemical analysis. Determination of plasma Total Protein (TP), albumin, Total Cholesterol (TC), creatinine (CR) hepatic enzymes activities (AST and ALT) and triglyceride (TG) were done colorimetrically by using available commercial kits (Diamond Diagnostics Company). Globulin (G) values were calculated by subtracting the values of albumin from the corresponding values of TP. Moreover, carcass traits as a percent of live body weight were calculated and small intestine length (cm) was measured.

Microbiological count and enzymatic activity determination: From each euthanized bird, abdominal cavity was opened and the total gastrointestinal tract immediately outspreaded, then, the ileum, starting from the Meckel's diverticulum to 4 cm above the ileo-caecal junction, was quickly dissected and the digesta contents of this intestinal segment (1 g) were collected and homogenized with 10 mL phosphate buffer solution (PBS in pH 7). The ileal digesta specimens were sent packed on ice to the laboratory (Microbiological Laboratory, MERCIN, Faculty of Agriculture, Ain Shams University) for enumeration of total bacteria, coliform and *Lactobacillus* counts. The intestinal length (cm) was also considered. The same located segments of their digestive tract (stomach and intestine) were emptied by gentle squeezing. Contents of individual segments were taken and mixed and about 1 g of the mixed content was immediately diluted with 10 mL of distilled water. All samples were centrifuged for10 min. The supernatant fluid was taken and stored in sealed bottles at -20°C until analyzed. Enzymes activity indigestive content of stomach and intestine of chicks were determined as follows: Amylase[12] and protease[13].

Histological examination of liver and ileum: Representative segments of small intestinal ileum and liver were taken from each euthanized bird, then subjected to fixation and histological segmentation as described by the method of Iji *et al.*[14] where, each segment was immersed in formaldehyde, before fixation in Bouin's solution and paraffin embedding. Paraffin sections at 6 µm thickness were made from each sample, stained with haematoxylin and eosin. Villus length was measured from the top of the villus to the top of the lamina propria and the crypt depth was measured from the base up to the region of transition between the crypt and villus according to Aptekmann *et al.*[15]. Then, the microscopic examination and photography for ileum and liver segments was carried out using advanced light microscope (Leica®, DM 2500, Germany). Villus length (µm) and crypt depth (µm) were analyzed and measured from each preparation using linear scaled graticule (10x) and combined LEICA, DM 2500 computerized software.

Statistical analysis: Data of chick performance, carcass traits, plasma constituents, microbial counts (cfu log/g), enzyme activity and histological aspects were statistically analyzed using SAS software[16] with one way analysis procedure and differences between means were separated using Duncan's multiple range test[17] at significance level 0.05. The statistical model performed as follow:

$$Y_{ij} = \mu + T_i + E_{ij,}$$

Where:

Y_{ij} = The experimental observation
μ = Overall mean
T_i = The effect of ith dietary treatment
E_{ij} = Random error

RESULTS AND DISCUSSION

Effect of experimental treatments on performance traits: The effect of dietary treatments on live Body Weight (BW), Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversion Ratio (FCR) through different experimental periods are shown in Table 2. It indicated that neither dietary supplementation with tested growth promoter sources nor

Table 2: Effect of experimental treatments on performance of broiler chicks at different periods

Items	Experimental treatments						
	Control	PL1.5	PL3.0	PO 125	PO250	AGP	Significance[#]
Starter period 7-21days old							
BW at 21 days old	882	865	882	884	866	847	NS
BWG (g) chick	839	822	839	840	822	803	NS
FI (g) chick	1060	1090	1103	1065	1060	1041	NS
FCR (g) chick/feed gain	1.263^{c}	1.326^{a}	1.315^{a}	1.268^{c}	1.290^{bc}	1.296^{b}	*
Grower period 22-35 days old							
BW at 35 days old	1846	1756	1851	1768	1848	1808	NS
BWG (g) chick	963	890	968	884	982	961	NS
FI (g) chick	1730	1612	1713	1630	1610	1568	NS
FCR (g) chick/feed gain	1.796^{abc}	1.811^{ab}	1.769^{abc}	1.843^{a}	1.639^{bc}	1.631^{c}	*
Overall period 7-35 days old							
BWG (g) chick	1802	1713	1808	1725	1805	1765	NS
FI (g) chick	2791^{a}	2703^{ab}	2816^{a}	2696^{ab}	2671^{ab}	2609^{b}	*
FCR (g) chick/feed gain	1.549^{ab}	1.578^{a}	1.558^{ab}	1.563^{ab}	1.480^{b}	1.478^{b}	*

[#a,b,c]Means within the same row with different superscripts are significantly differed, NS: Non significant difference, *$p \leq 0.05$

those levels did significantly affect BW, BWG or FI during starter and grower periods or BWG throughout the overall period, while FCR measurement was significantly affect for all experimental periods as well as FI during overall period. The PO125 treatments reported the best FCR values (1.263 and 1.268, respectively) at starter period, while, the worst FCR clearly recorded with both the two PL levels, a large part of this higher FCR values attributed to higher FI. Concerned result regarding to period from 7-21 days old in present study is contradict with that settled by Ocak *et al*.[4] as that broiler chicks fed PL diet grew faster than control group only from 7-21 days old indicating that peppermint leaves had a growth promoter effect on younger broiler chicks than older ones. But inconsistently, current study indicated that FCR and BWG at this early stage reported worst values with PL supplemented groups than control, PO or AGP treatments. In this study, the fiber content of PL which extremely absent in PO and AGP may be resulted in higher feed consumed by chicks fed PL treatments compared with control, PO or AGP treatments. This suggestion is agreement with many studies workers such as, Anderson *et al*.[18] and Melkamu[19] stated the presence of dietary fiber in broiler chicks resulted in higher feed intake but didn't support BWG or FCR, similarly, BWG didn't significantly affect while FCR negatively affect in present study.

Additionally, the figure positively converted to the side of AGP and PO250 supplemented diets during grower and overall periods, where both exhibited, significant better FCR (1.631 and 1.639, respectively), hence, caused a best FCR values at overall period that recorded 1.478 and 1.480, respectively. This improvement of FCR at both treatments largely attributed to the lower FI during both periods (grower = 1568 and 1610 g, overall = 2609 and 2671 g, respectively) not to BWG. Later results, suggests that the dietary supplementation of broiler chicks with 1 g antibiotic (flavomycin) or 250 mg PO kg^{-1} diet, caused a significant better FCR through grower and overall periods compared to treatments of control, PL1.5, PL3.0 or PO125. Unsufficient research publications had been investigated the effect of PO incorporation in broiler diets in single form (not as essential oil blend) on broiler performance traits, however, many published studies had been investigated the effect of dietary supplementation of peppermint leave and tested antibiotics on FI, BWG or FCR of broiler chicks and gave somewhat similar results. Among those; Sharifi *et al*.[20] when concluded that flavomycin or PL supplemented to diets by 0.3% rate significantly increased FI and BWG and improved FCR of broiler chicks compared with negative control group then they suggested that PL could be an alternative to antibiotic in diets for improving chick performance. By supplementing diets of broiler chicks with 0.50-1.5% PL; Galib and Al-Kassie[21] revealed that all performance traits (significantly improved) of chicks fed supplemented diets than un-supplemented group and 0.50% level was more enhancer than 1.5% level and a similar BWG and FCR improvement associated with PL supplementation in diet through 35 days old was confirmed by Cross *et al*.[22] and Ocak *et al*.[4].

Present study could pointed out to that; although it was expected that supplementing the dietary herbs[23] or plant extracts[24,25] would stimulate the growth performance of chick but the non significant effect on performance parameters in current study are contradict with studies of Alcicek *et al*.[10], Bampidis *et al*.[23] and Griggs and Jacob[26] using different herbs, plant extracts, essential oil and/or the

Table 3: Effect of experimental treatments on some carcass characteristics of broiler chicks at 35 days old

	Experimental treatments						
Items	Control	PL1.5	PL3.0	PO125	PO250	AGP	Significance[#]
Live body weight (g)	1901	1838	1940	1823	1902	1858	NS
Items as a percent of LBW							
Carcass	68.67	68.93	70.14	68.94	67.60	69.00	NS
Abdominal fat	1.187	0.730	1.087	0.820	0.987	0.902	NS
Liver	2.672	2.310	2.257	2.612	2.272	2.262	NS
Gizzard	1.252^{b}	1.377^{ab}	1.580^{a}	1.117^{b}	1.147^{b}	1.125^{b}	*
Heart	0.515	0.602	0.522	0.527	0.595	0.525	NS
Spleen	0.130	0.115	0.095	0.107	0.105	0.107	NS
Giblets[##]	4.440	4.297	4.360	4.260	4.017	3.917	NS
Bursa	0.072	0.077	0.080	0.080	0.117	0.087	NS
Small intestine length (cm)	136.2^{bc}	128.2^{bc}	140.2^{abc}	124.7^{c}	156.7^{a}	146.7^{ab}	*

[#a,b,c]Means within the same row with different superscripts are significantly differed, NS: Non significant difference, *p≤0.05, [##]Giblets: Sum weights of liver, gizzard, heart and spleen

main components of the essential oils. However, the results of the present study are in agreement with previous observations of Hernandez *et al.*[27] and Bampidis *et al.*[23], using herbs, plant extracts, essential oil and/or the main components of the essential oil that did not affect body weight gain or feed intake but could improve feed efficiency in broilers than those fed AGP.

As a general conclusion for chick performance in concerned study, both control and PO125 treatments revealed the best FCR among tested growth promoters through starter stage, while FCR exhibited better values with PO250 and AGP groups during grower and overall periods, compared to control, PL and PO125 treatments.

Effect of experimental treatments on carcass traits: Table 3 displays the effect of experimental treatments on carcass traits at 35 days old and summarized to that, the carcass percent and most carcass parts didn't significantly differed between experimental treatments, except gizzard percentage that was exhibited higher values with the two levels of dietary PL compared with control, PO125, PO250 or AVP groups. The length of small intestine showed a non significant difference between treatments without certain trend.

The non-significant effect on carcass and most carcass parts is consistent with Ocak *et al.*[4] and Galib and Al-Kassie[21] using PL while higher gizzard percent associated with PL treatments compared with control, PO or AGP groups may be attributed to the relative high fiber content of PL compared with the other tested growth promoters and control diet, where many research workers involving Gonzalez-Alvarado *et al.*[28], Amerah *et al.*[29] and Svihus[30] reported a positive relationship between gizzard weight and fiber content of chicken diets. Although, Sklan *et al.*[31] and Amerah *et al.*[29] documented that the higher gizzard percent for PL treatments is resulted in increasing fiber content of broiler should be accompanied with higher length of small intestine but, unlike that the unclear trend of significant difference between treatments for small intestine length, calling for publish more studies interested to this point. However, broiler chicks had numerically more intestinal length and gizzard percent when fed diets supplemented with PL or PO in comparison with the control and somewhat AGP group birds.

Effect of experimental treatments on essential blood components: Results for Total Proteins (TP), Albumin (A), Globulin (G), A:G ratio, total cholesterol (TCH), creatinine (CR) and ALT, AST of chick plasma at 35 days old are presented in Table 4. Dietary AGP and the two levels of PO tended to increase concentrations of TP and G while no significant differences were found for Albumin values.

Dietary supplementation with PL1.5, PO125, PO250 and AGP, resulted in lower A: G ratio than control (4.14) and PL3.0 (2.05) and ranged between 1.14 and 1.20. On the other hand, total cholesterol in plasma for both control and AGP groups was lower compared to all peppermint treatments ($p<0.05$). Even thought the A/G ratio didn't showed significant difference between treatments, but, the two levels of PO exhibited lower A/G (PO125 = 1.14 and PO250 = 1.19) followed by AGP (1.20). According to the antagonistic relationship between plasma A/G ratio and body immunity status as settled by El-Agib *et al.*[32], later result suggested to that, there is a higher immunity property for the three growth promoter materials compared to control diet (2.05) or PL (PL1.5 = 1.70 and PL3.0 = 4.14). The immune- stimulatory property of many some commercial antibiotic preparations for broiler chicks was confirmed by Lee *et al.*[33], while, reviews interested to this point for phytobiotics and/or those essential oils are very lake.

Table 4: Effect of experimental treatments on some plasma constituents of broiler chicks at 35days old

	Experimental treatments						
Items	Control	PL1.5	PL3.0	PO125	PO250	AGP	Significance#
TP (g dL^{-1})	6.43[ab]	6.94[a]	5.26[b]	7.36[a]	6.94[a]	7.35[a]	*
Albumin (g dL^{-1})	4.15	4.14	3.89	3.88	3.62	3.94	NS
Globulin (g dL^{-1})	2.28[ab]	2.80[a]	1.37[b]	3.47[a]	3.33[a]	3.40[a]	*
A/G ratio	2.05	1.70	4.14	1.14	1.19	1.20	NS
Creatinine (mg dL^{-1})	1.29	1.01	1.67	2.06	0.95	0.89	NS
Total cholesterol (mg dL^{-1})	167[b]	164[b]	192[ab]	235[a]	169[b]	176[b]	*
AST u100 mL^{-1}	40.9	38.8	42.5	43.6	47.6	45.0	NS
ALT u100 mL^{-1}	17.20[b]	16.89[b]	17.63[b]	17.51[b]	17.03[b]	26.04[a]	*

[#a,b,c]Means within the same row with different superscripts are significant1y differed, NS: Non significant difference, *$p \leq 0.05$

Table 5: Effect of experimental treatments on different bacterial counts (log cfu$\times 10^5$ g^{-1}) of small intestine of broiler chicks at 35 days old

	Experimental treatments						
Items	Control	PL1.5	PL3.0	PO125	PO250	AGP	Significance#
Total count	6.69	6.44	6.57	6.45	6.73	6.37	NS
Coliform count	7.12	6.86	6.44	6.83	6.92	7.06	NS
Lactobacillus count	2.00	4.13	4.48	4.70	3.33	3.43	NS

#: Significance and NS: Not significant difference

The negative effect of antibiotic on plasma TCH may resulted from its inhibition impact on the culture within the intestine as reported by Francis *et al.*[34] or to that AGP caused deconjugation of bile salts in the intestine, thus preventing them from acting as precursors in TCH synthesis[35]. When concerning to plasma indicators for kidneys function and liver, the results of this study indicated that, no significant differences were found for either creatinine or AST between supplemented and un-supplemented groups. A significant increase was recorded for ALT in AGP group than the case of all peppermint extract treatments and control group.

Effect of experimental treatments on common beneficial and pathogenic bacteria counts of small intestine: The effect of dietary supplementation with different growth promoter types on microflora accounts (log CFU$\times 10^5$ g^{-1}) of small intestine of broiler chicks at 35 days old are documented in Table 5, which revealed a non significant difference among the dietary groups for the total bacteria count of small intestine. The highest numbers of coliform bacteria were found in the content of intestines from birds fed control group (7.12 CFU$\times 10^5$) and in those fed AGP supplemented diet (7.06 CFU$\times 10^5$). On the other hand, the results for *Lactobacillus* bacteria from groups fed with PL or PO proved high lactobacilli count of small intestine ranged from 4.48-4.70 CFU$\times 10^5$ and the control group recorded the lowest count (2.00 CFU$\times 10^5$), while, the AGP was moderated (3.43 CFU$\times 10^5$), accordingly, there are enough evidences those highlighted that plant extracts, essential oil and/or the main components of the essential oil may have a marked role in combating pathogenic bacteria populations in poultry[26]. The PL and PO treatments obviously achieved lower count of total coliform bacteria and higher count of lactobacilli and this result is agreed with Sharifi *et al.*[20]. Additionally, the PL3 significantly exhibited a lower counts of intestinal clostridia and bifidobacteria similar for flavomycin treatment. However, the antibacterial activity effect of PL and PO for pathogenic bacteria comes from that PL and PO contains varied levels of monoterpens, thymol and menthol components[6], where the monoterpen residues as settled by Kotana *et al.* [36] and Abdel-Wareth[37] have antibacterial activity for this type of bacteria as a result of its lipophilic property, hence, penetrate the cell membranes and mitochondria of the microorganisms then inhibited membrane bound electron flow causing inhibition of energy metabolism of bacterial cell. Moreover, the menthol have a considerable microbial ecosystem effect on intestinal gut as documented by Williams and Losa[38] and Cross *et al.*[22]. Additionally, Trombetta *et al.*[39] confirmed that both thymol and menthol and most terpen forms have inhibitory effect on pathogenic bacteria by crossing the cell membranes, penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activity. As a general antibacterial effect of the components of PL and PO causing a desirable and positive effect on the health of intestinal tract of bird. While, the mode of action for the positive effect of those compounds on *Lactobacillus* bacteria in previous studies have not be sufficiently investigated but current study suggested those compounds may reducing the intestinal PH media causing activation of *Lactobacillus* bacteria that able to

Table 6: Effect of experimental treatments on amylase and protease levels of stomach and ileum of broiler chicks at 35 days old

	Experimental treatments						
Items	Control	PL1.5	PL3.0	PO125	PO250	AGP	Significance[#]
Stomach amylase	0.273[e]	0.923[d]	2.493[c]	2.310[c]	3.443[b]	7.043[a]	*
Stomach protease	9.54[e]	19.45[c]	30.33[b]	30.37[b]	40.65[a]	16.00[d]	*
Ileum amylase	31.39[e]	58.39[c]	75.15[b]	75.20[b]	90.53[a]	40.38[d]	*
Ileum potease	6.66[e]	9.45[c]	12.13[b]	12.14[b]	15.76[a]	8.06[d]	*

[#a,b,c]Means within the same row with different superscripts are significantly differed, *p≤0.05

Table 7: Effect of experimental treatments on villus length (VL) and crypt of lieberkühn depth (CrD) of intestinal ileum segments of broiler chicks at 35 days old

	Experimental treatments					
Items[#]	Control	PL1.5	PL3.0	PO125	PO250	AGP
Villus length (μm)	702.9[b]	1355.2[a]	831.5[a]	699.7[b]	1072.0[a]	722.0[b]
Crypt of lieberkühn depth CrD (μm)	140.0[b]	190.4[a]	181.4[a]	181.2[a]	219.1[a]	121.9[b]

[#]Represents the average for observations of 10 segments each treatment

markedly inhibit growth of pathogenic bacteria of intestinal gut by many mechanisms including stimulation of adaptive immunity and/or production of inhibitory metabolites, such as organic acids as suggested by Neal-McKinney *et al.*[40]. However, even thought, all peppermint treatments in concerned study statistically exhibited a non-significant difference with control and AGP treatment for tested bacterial counts of ileum but, those resulted in a higher count of *Lactobacillus* and lower coliform count compared with control and antibiotic-supplemented diets, indicative they improves the microbial media of intestinal gut.

Effect of experimental treatments on amylase and protease activity of stomach and ileum: Digestive enzymes activities (amylase and protease) in different segments of both stomach and ileum are presented in Table 6. The AGP recorded the highest stomach amylase level among treatments, additionally, compared with control and AGP treatments; all peppermint extract supplemented diets recorded significant highly stomach amylase and ileal amylase and protease especially for PO250 group, indicated that PL and PO may have a positive stimulation for secretion of endogenous digestive enzymes, this result is in harmony with Ocak *et al.*[4] for peppermint extracts and Cross *et al.*[22] for several types of tested essential oils in broiler chick diets. Similarly, Abdelaziz *et al.*[41] reported a significant higher activity of ileum amylase and protease of broiler chicks fed diets supplemented with PL compared with control group.

Accordingly, the tested peppermint extracts in current study exhibited positive and enhancement effect on the stomach protease and ileum amylase and protease and those supports the digestion of essential nutrients, furthermore, this effect may be more obvious than tested antibiotic (AGP). While, AGP increased only stomach amylase compared with peppermint and control groups, suggesting that, both peppermint extracts (PL and PO) and tested antibiotic (AGP) have a competitive impact for this issue.

Effect of experimental treatments on measured traits and some histological examinations of small intestine and liver segments: Measured traits and histological examination and of the small intestinal and liver sections are illustrated in Table 7 and Fig. 1 and 2. Concerning to the intestinal sections and its Villus Length (VL)and crypt of lieberkühn depth (Cr D). It is clear that PL1.5, PL3.0 and PO250 recorded the highest VL among treatments (ranged between 831.5-1355.2 μm vs the values of 702.9, 699.7 and 722.0 μm for control, PO125 and AGP groups, respectively). Additionally, the same treatments besides PO125 group recorded the higher Cr D (from 181.2-219.1 μm) compared to the lower Cr D for control (140.0 μm) and AGP supplemented diet (121.9 μm).

Respecting to this result, the earlier studies for Caspary[42] and Anonymous[43] referred to that, higher length of intestinal microvilli potentially increased surface area capable of greater absorption of available nutrients, besides, that the villus crypt seemed to be as the villus factory and deeper crypts indicated a fast tissue turnover to renewal of the villus as urgent need under conditions of normal sloughing or inflammation from pathogens or their toxins and high demands for tissue, thereupon and according to current study, the PO250 treatment had a considerable positive effect on histological profile of the main absorptive components of small intestine causing healthy-intestinal gut and higher absorptive capability. The main effect of this result may be due to the enhancer effect of thymol compound present in many parts of peppermint plant (with variable rate between parts) and consequently its extracts. This result and a similar suggestion is in agreement using different photogenic sources of thymol

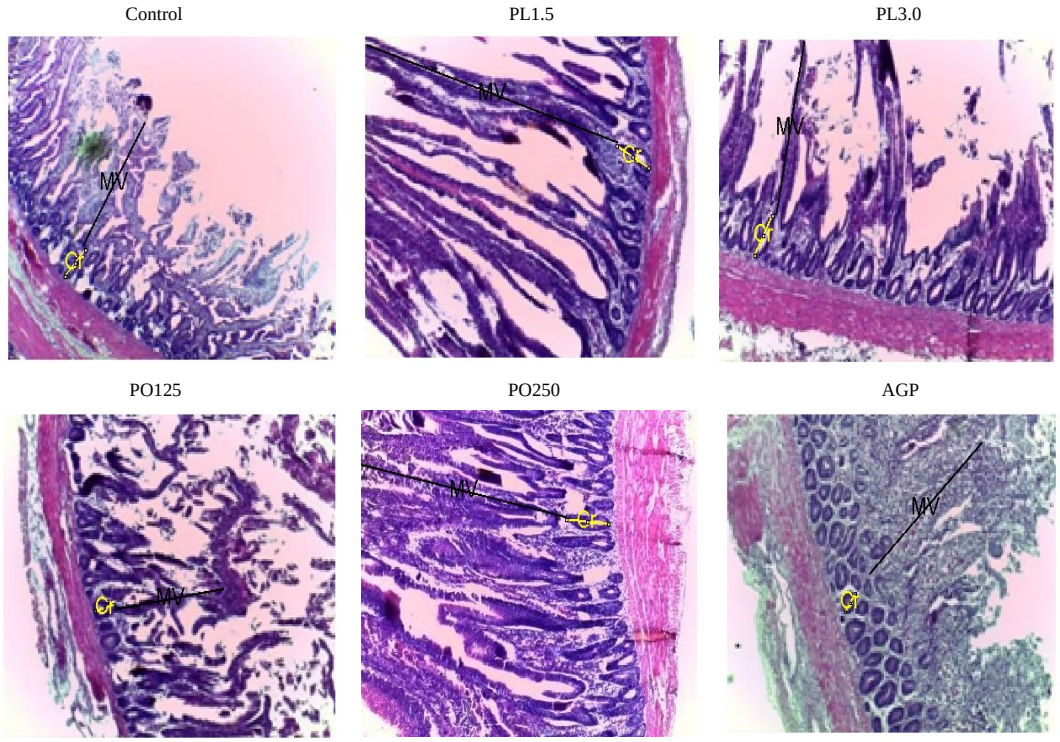

Fig. 1: Histological structure (at 10X) of the small intestine from broiler chicks at 35 days old fed different experimental treatments.*MV (dark line): Microvilli and Cr (light line): Crypt of lieberkühn

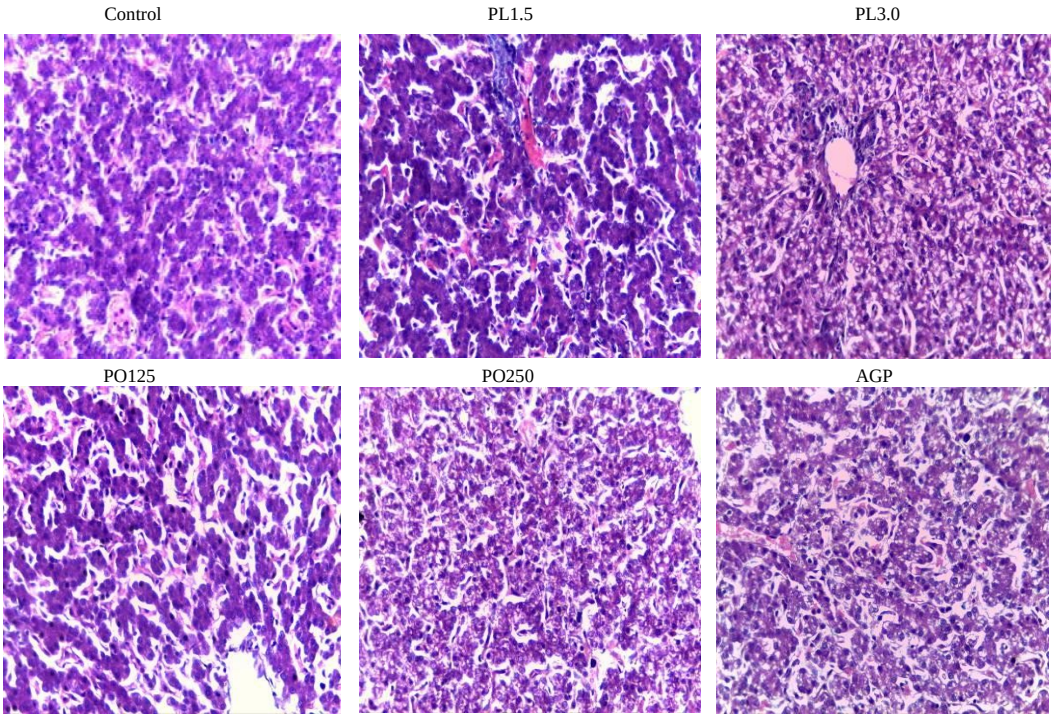

Fig. 2: Histological structure (at 10X) of the liver from broiler chicks at 35 days old fed different experimental treatments

like oregano oil with Vazquez *et al.*[44], dried extracts of oregano, cinnamon and pepper in study of Hernandez *et al.*[27] and peppermint leaves or thyme of Ocak *et al.*[4] study.

On the other hand, the sections from liver parenchyma of control treatment (Fig. 2) have normal hepatocytic structure with dilated central vein engorged with blood. Also, there were dark stained eosinophilic cells surrounding or near the central veins. There is moderate hypertrophy of liver cells especially in AGP group which may reveal hyperactivity of the liver cells or a compensatory effect due to more degenerative (necrotic) are a synthesis sections. Those changes in liver sections may be related to the higher metabolic activity associated with the higher growth rate of broilers that basically depends on their genetic background. Unfortunately, there is a lake in the study information interested to the effect of peppermint extracts, main peppermint components (thymol, menthol and terpenes) or AGP on liver histology in broiler chicks but, generally, concerned study indicated that both peppermint extracts have a desirable effect on the main absorptive cells of mucosal layer of intestinal ileum of broiler chicks besides some histological changes of the liver tissue and elicit a call for more investigation.

CONCLUSION

The results of concerned study could be summarized to the following points. Supplementation of broiler diets with Peppermint Oil (PO) by 125 mg kg^{-1} (PO125) significantly improved Feed Conversion Ratio (FCR) from 7-21 days old compared with control, PO with 250 mg kg^{-1} (PO250), 1.5 g and 3.0 g peppermint leaves (PL 1.5 and PL3.0) kg^{-1} diet or 1 g flavomycin (AGP) kg^{-1} diet. While, FCR for both PO250 and AGP groups performed a better FCR during grower and overall periods compared the other treatments. Experimental treatments didn't significantly influence the most carcass traits, except gizzard percent, that increased with all peppermint extract treatments. Compared with un-supplemented diet; all supplemented diets increased plasma total protein, total cholesterol and globulin as well as affect plasma liver enzymes but with unclear trend between treatments. Both PO levels and AGP slightly improved the immunity indicators of chick plasma. All peppermint extracts tended to improves microbial populations in intestinal ileum and enhances stomach protease and ileum amylase and protease and the latter figure was more cleared than tested antibiotic (AGP) which increased only the stomach amylase, calling to suggest that, tested peppermint extracts and antibiotic have a competitive impact for this manner. A desirable effect on villus length and crypt depth of intestinal ileum was accompanied with supplementation of diets with peppermint extracts compared with control or antibiotic diets, besides that, all tested growth promoters exhibited some histological changes of the liver tissue. Supplementation of broiler chick diets with peppermint oil by 250 mg kg^{-1} significantly achieved the best effects for most measurements included in current study compared with control diet, peppermint leaves with 1.5 and 3.0 g levels, 125 mg peppermint oil kg^{-1} diet or 1 g flavomycin kg^{-1} diet.

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