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Studies on supplementation of peppermint essential oil and organic acids on performance and gut health of broilers

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Abstract

The present study was conducted to evaluate effect of supplementation of peppermint essential oil and organic acids on performance and gut health of broilers. The present research work was conducted on 420 Vencobb-400 straight-run broiler chickens. The day old chicks were randomly distributed into 8 treatment groups and each treatment group had 3 replicates of 20 chicks each. The control group (A) received basal diet and the different treatment groups viz. Group B received a basal diet supplemented with peppermint essential oil at 200 mg/kg, Group C, D, E received a basal diet supplemented with 1% sorbic acid, fumaric acid and propionic acid respectively. Whereas, group F, G and H received a combination of peppermint essential oil at 200 mg/kg with 1% sorbic acid, fumaric acid and propionic acid, respectively. At the end of the study, body weight and weight gain were improved in birds supplemented with a combination of peppermint essential oil with sorbic acid as compared to control and other treatment groups. The supplementation of essential oil and organic acids either singly or in combination showed significantly ($P < 0.01$) better FCR compared to control group at the end of 6th week of age. The TVC and *E. coli* count were significantly ($P < 0.01$) reduced in treatment groups compared to control. The supplementation of combination of peppermint essential oil with sorbic acid was found most beneficial to enhance growth performance and gut health in broiler production.

Keywords: Organic acid, peppermint essential oil, performance, broiler, gut health

Introduction

For improving growth performance in poultry, the antibiotics have been used as feed supplements [1, 2]. Antibiotics are helpful which prevents some specific pathogenic microorganism. However, recently antibiotics used as growth promoters in poultry feeds have been banned [3]. There is the development of antibiotic resistant for human pathogenic bacteria. Hence, there is need of researching new natural alternative additives instead of antibiotics in poultry feed [4]. The essential oils are volatile oils present in different herbs. The essential oils are known to promote feed intake, digestive enzyme production. It also enhances the immune system, and helpful in destroying pathogens. Essential oils being phenolic compounds, they act as antibacterial as phenols increase cell permeability, which results in water imbalance and cell death. Because of this, bacterial resistance to phenols cannot develop. The essential oil extracted from aromatic plants has been shown antibacterial, anticoccidial [5], antifungal [6] and antioxidant [7] activities. Another alternative to antibiotic growth promoters are organic acids which have shown positive results in poultry production. For reducing the intestinal pH and bacterial growth intolerant to pH changes [8, 9], thus providing better intestinal health for the bird to obtain maximum nutrient absorption. The plan of combining essential oils and organic acids is proving to be effective because there appears to be a fusion effect between the two concepts. At high pH levels the essential oils enable the anions of organic acids to penetrate the bacterial cell wall and disturb the metabolic processes in the bacterial cell. The application of proper combination of certain kinds of organic acids and essential oil in poultry nutrition could be a promising alternative to antibiotic growth promoters in broilers. Keeping these facts in view, the present experiment was planned to evaluate combination of sorbic acid, fumaric acid and propionic acid with peppermint essential oil to improve growth performance in broiler chickens.

Materials and methods

The experiment was conducted on 480 straight-run commercial day-old broiler chicks of Vencobb-400 strain for 6 weeks. The chicks were randomly distributed into eight dietary treatments groups with three replicates of 20 birds

each. The rations were formulated for three phases viz. pre-starter (0-7 days), starter (7-21 days) and finisher for all treatment groups as per BIS (2007) [10]. The details of the dietary treatments are given below-

Table 1: The details of different dietary treatments using combination of Peppermint Essential oil with different organic acids

Treatment group	Dietary treatment details	No. of birds/replicate	Replicates / treatment	No. of birds/ Treatment
A	Maize soya based control diet	20	3	60
B	Basal diet + 200 mg/kg diet Peppermint Essential oil	20	3	60
C	Basal diet + Sorbic acid 1%	20	3	60
D	Basal diet + Fumaric acid 1%	20	3	60
E	Basal diet + Propionic 1%	20	3	60
F	Basal diet + 200 mg/kg diet Peppermint Essential oil + Sorbic acid 1%	20	3	60
G	Basal diet + 200 mg/kg diet Peppermint Essential oil+ Fumaric acid 1%	20	3	60
H	Basal diet + 200 mg/kg diet Peppermint Essential oil+ Propionic acid 1%	20	3	60

Measurements

Birds from each group were weighed individually on day 0 and at weekly intervals. Mean live body weight gain (g/ b) was computed on weekly basis. Measured quantity of feed was offered every day and the left over feed was recorded. The difference between the feed offered and balanced feed was worked out to know the actual feed consumed by each group during each week. Live body weight, body weight gain and the feed intake of chicks allotted in replicates were recorded at weekly interval up to 6 weeks of age. The feed conversion ratio was calculated on the basis of unit feed consumed to unit body weight gain for each replicate separately. Mortality was recorded as and when occurred.

Total viable count and *E. coli* count

The total viable count and Coli form count in all the treatment groups was studied at 21st day age following standard laboratory procedures. For determination of total viable count and coliform count one bird from each replicate was sacrificed by severing the jugular vein and carotid artery on one side of neck and allowed to bleed for 1 to 2 minutes. Large intestine was opened immediately after sacrificing and weighed 1 g of ceacal contain collected in sterile glass vial diluted in 9 ml Normal saline and then 2 serial dilutions were made for inoculation. Then, 10 μ l content from the last test tube was poured on the Macconky's media plate and kept for incubation for 24 hrs at 37 °C. After incubation, total bacterial colonies were counted as colony forming units (CFU /per gram) of samples [11]. The average number of colonies in a

particular dilution was multiplied by the dilution factor to obtain the total viable count. The total viable count was calculated according to ISO (1995) [12]. The results of the total bacterial count were expressed as the number of organism of colony forming units per gram (CFU/gm) of ceacal samples.

Statistical analysis

The differences among treatment groups were determined by analyzing the data generated, by using Complete Randomized Design [13]. The treatment means were compared by critical differences (CD) and Analysis of Variance.

Results and discussion

Body weight and body weight gain

The initial body weights of broiler chicks were almost same in all dietary treatments indicating the treatment groups were homogenous in nature. The analysis of variance for live body weight showed significant differences among treatment groups at 3rd and 4th weeks of age (Table 1). The broilers supplemented with peppermint essential oils and sorbic acid resulted into significantly ($P<0.05$) higher body weights compared to control group at 3rd and 4th weeks. There were non-significant differences for mean weekly live body weights in all treatment groups at 1st, 2nd, 5th and 6th weeks of age. At the end of sixth week the highest body weight was observed in birds supplemented with peppermint essential oil and sorbic acid, however, the difference was statistically non-significant.

Table 1: Weekly live body weight (g/b) of broilers fed with Peppermint Essential oil and different organic acids.

Treatment groups	Age (weeks)						
	Day-old	1 st	2 nd	3 rd	4 th	5 th	6 th
A	45.03 \pm 0.83	144.49 \pm 1.97	378.22 \pm 0.97	739.74 ^c \pm 7.03	1190.53 ^c \pm 20.37	1740.50 \pm 15.57	2343.04 \pm 56.84
B	44.23 \pm 0.19	146.98 \pm 3.20	383.12 \pm 7.57	781.25 ^b \pm 7.74	1285.96 ^b \pm 10.26	1868.63 \pm 31.65	2431.07 \pm 24.53
C	44.38 \pm 1.42	149.19 \pm 1.77	391.52 \pm 9.80	798.21 ^b \pm 23.79	1294.99 ^{ab} \pm 26.97	1858.48 \pm 51.31	2477.87 \pm 52.17
D	43.45 \pm 0.26	148.15 \pm 0.95	396.65 \pm 0.60	789.68 ^b \pm 8.33	1283.81 ^b \pm 35.35	1830.59 \pm 24.89	2429.82 \pm 63.01
E	44.78 \pm 1.16	148.12 \pm 0.82	379.47 \pm 5.23	770.48 ^{bc} \pm 10.97	1293.18 ^{ab} \pm 3.18	1857.62 \pm 23.43	2489.83 \pm 15.83
F	45.18 \pm 0.67	149.59 \pm 3.87	391.91 \pm 1.77	855.89 ^a \pm 0.68	1355.16 ^b \pm 24.77	1901.83 \pm 26.06	2604.10 \pm 64.25
G	44.55 \pm 0.52	145.83 \pm 3.91	379.01 \pm 2.84	783.28 ^b \pm 12.56	1319.20 ^{ab} \pm 25.60	1853.52 \pm 41.82	2542.29 \pm 75.52
H	44.10 \pm 0.88	146.93 \pm 2.09	378.08 \pm 6.84	787.49 ^b \pm 2.25	1289.59 ^{ab} \pm 11.85	1844.62 \pm 28.93	2490.00 \pm 50.94
CD	NS	NS	NS	46.827** 33.986*	91.395 ** 66.332*	NS	NS
CV%	3.273	3.036	2.440	2.491	2.973	3.027	4.439

Means bearing different superscripts within a column differ significantly. * $P<0.05$, ** $P<0.01$, NS-Non-significant.

Table 2: Analysis of variance for weekly live body weight (g/b) of broilers fed with Peppermint Essential oil and different organic acids.

Source	df	Day old		1 st week		2 nd week		3 rd week	
		MSS	'F' value	MSS	'F' value	MSS	'F' value	MSS	'F' value
Treatments	7	0.93	0.44	8.74	0.44	166.85	1.89	3179.89	8.25
Error	16	2.12		20.03		88.15	-	385.49	
		4 th week		5 th week		6 th week			
		MSS	'F' value	MSS	'F' value	MSS	'F' value		
Treatments	7	6460.70	4.40	6568.56	2.11	18438.03	2.12		
Error	16	1468.49	-	3116.63	-	8700.47	-		

MSS - Mean sum of squares, df - Degrees of freedom, CD-Critical difference, CV-Coefficient of variance.

The analysis of variance for cumulative weight gain showed significant differences among the treatment groups at 3rd and 4th weeks of age (Table 3). The birds fed peppermint essential oil and sorbic acid recorded significantly ($P < 0.05$) higher cumulative weight gain as compared to control group at the age of 3rd and 4th week. All the treatment groups supplemented either with essential oil, organic acids or combination of essential oils and organic acids recorded

significantly ($P < 0.05$) higher cumulative weight gain as compared to control group. At 1st, 2nd, 5th and 6th weeks of age, the cumulative weight gain was non-significant in all treatment groups. At the end of sixth week, all treatment groups showed numerically higher cumulative weight gain but the statistical difference was non-significant. However, birds fed peppermint essential oil and sorbic acid recorded highest cumulative weight gain.

Table 3: Cumulative weekly weight gain (g/b) of broilers fed with Peppermint Essential oil with different organic acids.

Treatment groups	Age (weeks)					
	1 st	2 nd	3 rd	4 th	5 th	6 th
A	99.45 ± 2.43	333.19 ± 1.42	694.71 ^c ± 6.29	1145.50 ^c ± 20.34	1695.47 ± 15.48	2298.01 ± 57.27
B	102.75 ± 3.01	338.88 ± 7.41	737.02 ^b ± 7.60	1241.72 ^b ± 10.15	1824.39 ± 31.75	2386.84 ± 24.70
C	104.81 ± 3.08	347.14 ± 11.22	753.83 ^b ± 25.19	1250.61 ^{ab} ± 28.08	1814.10 ± 52.43	2433.49 ± 53.57
D	104.70 ± 0.92	353.20 ± 0.38	746.23 ^b ± 8.09	1240.36 ^b ± 35.23	1787.14 ± 24.95	2386.37 ± 63.08
E	103.33 ± 0.42	334.68 ± 5.48	725.70 ^{bc} ± 10.68	1248.40 ^{ab} ± 2.81	1812.83 ± 22.28	2445.05 ± 15.26
F	104.40 ± 3.32	346.73 ± 1.60	810.71 ^a ± 1.28	1309.98 ^a ± 25.40	1856.65 ± 26.72	2558.91 ± 64.92
G	101.28 ± 3.82	334.46 ± 2.88	738.73 ^b ± 12.03	1274.65 ^{ab} ± 25.09	1808.97 ± 41.41	2497.74 ± 75.10
H	102.83 ± 2.14	333.98 ± 6.68	743.39 ^b ± 1.81	1245.49 ^{ab} ± 12.62	1800.52 ± 29.81	2445.90 ± 51.54
CD	NS	NS	47.575** 34.523*	92.237 ** 66.938*	NS	NS
CV%	4.439	2.945	2.689	3.101	3.121	3.852

Means bearing different superscripts within a column differ significantly. * $P < 0.05$, ** $P < 0.01$, NS-Non-significant.

Table 4: Analysis of variance for cumulative weekly weight gain (g/b) of broilers fed with Peppermint Essential oil and different organic acids.

Source	df	1 st week		2 nd week		3 rd week	
		MSS	'F' value	MSS	'F' value	MSS	'F' value
Treatments	7	10.21	0.49	176.83	1.75	3168.32	7.96
Error	16	20.89	-	100.74	-	397.91	-
		4 th week		5 th week		6 th week	
		MSS	'F' value	MSS	'F' value	MSS	'F' value
Treatments	7	6461.01	4.32	6574.25	2.08	18371.71	2.08
Error	16	1495.50	-	3162.31	-	8805.84	-

MSS - Mean sum of squares, df - Degrees of freedom, CD-Critical difference, CV-Coefficient of variance.

The findings are in accordance with Al Kassie, [14] who reported that inclusion peppermint oil in broiler diet showed increased average gain in weight. Panda *et al.* [15] studied the effect of butyric acid supplementation in broiler ration at the dose level of 0.2, 0.4 and 0.6 percent and found improvement in body weight gain. Alcicek *et al.* [16] also reported that, supplementation of a blend of essential oil in broiler diet showed improved body weight. Results are also in agreement with Athina *et al.* [17] who recorded improved broiler chickens performance with the combined dietary supplementation with oregano, attapulgit and benzoic acid. Hassan *et al.* [18] found that addition of organic acid in the diet significantly resulted in more weight gain in broilers. However, supplementing essential oils or organic acids as a growth enhancer may not always improve production performance in broilers. Pirgozliev [8] reported that use of fumaric and sorbic acid as additives in broiler feed did not significantly affect weight gain or feed efficiency. Alaeldein *et al.* [19] observed that, addition of commercial essential oil blend, as an alternative to

antibiotic in-feed, body weight gain was not significantly different among all treatments ($P > 0.05$). Hernandez *et al.* [20] reported that fumaric acid (0.5% to 1%) did not affect body weight, body weight gain. Pappas *et al.* [21] found that addition of essential oil blend (EO) and benzoic acid in broilers did not significantly affect chicken body weight.

In general, essential oils and organic acids are potential growth promoters. The idea of combining essential oils and organic acids is beneficial as at high pH levels the essential oils enable the anions of organic acids to penetrate the bacterial cell wall and disturb the metabolic processes in the bacterial cell. The organic acids or their salts enhanced performance in the broilers may be due to improved protein and energy digestibility and reduces other growth suppressing microbial metabolites [22]. The beneficial impact of essential oils in broilers in the modulation of gut microbiota, improved nutrient utilization and growth performance [23]. In present study also the body weight and weight gain were improved in birds supplemented with combination of peppermint essential

oil with sorbic acid as compared to control and other treatment groups.

Weekly feed consumption

The analysis of variance for cumulative weekly feed consumption showed non-significant differences among the

treatment groups (Table 5). All the treatment groups showed lower cumulative feed consumption as compared to control group during entire experimental period. There were non-significant differences for cumulative feed consumption in all treatment groups at 6th week.

Table 5: Cumulative weekly feed consumption (g/b) of broilers fed with Peppermint Essential oil and different organic acids.

Treatment groups	Age (weeks)					
	1 st	2 nd	3 rd	4 th	5 th	6 th
A	128.08 ±9.81	422.13 ±6.92	978.74 ±21.35	1803.25 ±35.34	2888.94 ±29.94	4095.73 ±35.54
B	120.87 ±1.49	415.40 ±5.25	968.30 ±5.21	1772.48 ±5.98	2813.42 ±15.26	4004.82 ±30.11
C	122.68 ±1.65	414.89 ±11.47	975.08 ±22.34	1779.81 ±43.42	2806.60 ±88.47	3997.72 ±92.73
D	123.48 ±0.41	424.33 ±6.04	958.87 ±6.18	1758.94 ±37.08	2737.41 ±38.82	3900.27 ±43.65
E	121.02 ±2.30	406.60 ±3.65	938.85 ±10.63	1729.41 ±24.88	2779.42 ±15.64	4000.89 ±16.05
F	123.48 ±1.37	410.88 ±3.75	988.21 ±11.87	1794.04 ±82.24	2829.49 ±101.41	4031.81 ±115.64
G	119.20 ±4.35	400.11 ±9.75	938.98 ±11.43	1775.13 ±40.30	2766.02 ±82.20	3962.78 ±94.14
H	124.47 ±1.01	408.16 ±6.09	951.86 ±11.03	1737.72 ±25.97	2744.38 ±33.71	3949.52 ±34.94
CD	NS	NS	NS	NS	NS	NS
CV%	5.646	2.976	2.480	4.122	3.727	2.925

NS-Non-significant.

Table 6: Analysis of variance for cumulative weekly feed consumption (g/b) of broilers fed with Peppermint Essential oil and different organic acids.

Source	df	1 st week		2 nd week		3 rd week	
		MSS	'F' value	MSS	'F' value	MSS	'F' value
Treatments	7	22.03	0.45	195.36	1.30	1009.40	1.76
Error	16	48.16	-	151.09	-	572.40	-
		4 th week		5 th week		6 th week	
		MSS	'F' value	MSS	'F' value	MSS	'F' value
Treatments	7	1977.06	0.37	7476.49	0.70	10151.62	0.75
Error	16	5334.98	-	10831.92	-	13645.56	-

MSS - Mean sum of squares, df - Degrees of freedom, CD-Critical difference, CV-Coefficient of variance.

The present findings are in accordance with Adil *et al.* [24], who recorded lower cumulative feed consumption in the groups supplemented with organic acids compared to control group. Alciçek *et al.* [16] reported that dietary supplementation of the organic acid does not affect the feed intake at 21 and 42 days of age in broilers. Alaeldein *et al.* [19] also observed that feed intake was not significantly different among all treatments, with the addition of commercial essential oil blend, as an alternative to antibiotic in-feed.

Feed conversion ratio

From Table 7, it was observed that the analysis of variance for weekly feed conversion ratio showed significant ($P<0.01$) differences among all treatment groups at 3rd, 4th, 5th and 6th week of age. During these weeks, the mean weekly feed conversion ratio was significantly better ($P<0.01$) in all treatment groups as compared to control group. The supplementation of essential oil, organic acids or their combination showed significantly ($P<0.01$) better FCR compared to control group at the end of 6th week of age.

Table 7: Cumulative weekly feed conversion ratio of broilers fed with Peppermint Essential oil and different organic acids.

Treatment groups	Age (weeks)					
	1 st	2 nd	3 rd	4 th	5 th	6 th
A	1.29 ± 0.09	1.27 ± 0.02	1.41 ^a ± 0.02	1.57 ^a ± 0.04	1.70 ^a ± 0.003	1.78 ^a ± 0.03
B	1.18 ± 0.02	1.23 ± 0.02	1.31 ^b ± 0.01	1.43 ^b ± 0.02	1.54 ^b ± 0.02	1.68 ^b ± 0.01
C	1.17 ± 0.02	1.20 ± 0.03	1.29 ^b ± 0.02	1.42 ^b ± 0.01	1.55 ^b ± 0.01	1.64 ^b ± 0.03
D	1.18 ± 0.01	1.20 ± 0.02	1.29 ^b ± 0.01	1.42 ^b ± 0.02	1.53 ^b ± 0.01	1.64 ^b ± 0.03
E	1.17 ± 0.03	1.22 ± 0.02	1.29 ^b ± 0.01	1.39 ^b ± 0.02	1.53 ^b ± 0.01	1.64 ^b ± 0.01
F	1.19 ± 0.04	1.19 ± 0.01	1.22 ^c ± 0.01	1.37 ^b ± 0.07	1.53 ^b ± 0.06	1.58 ^b ± 0.07
G	1.18 ± 0.08	1.20 ± 0.04	1.27 ^b ± 0.01	1.39 ^b ± 0.02	1.53 ^b ± 0.02	1.59 ^b ± 0.02
H	1.21 ± 0.02	1.22 ± 0.04	1.28 ^b ± 0.02	1.40 ^b ± 0.02	1.52 ^b ± 0.01	1.62 ^b ± 0.03
CD	NS	NS	0.063 ** 0.046*	0.128 ** 0.093 *	0.107** 0.073*	0.101*
CV%	6.902	3.822	2.032	3.767	2.798	3.550

Means bearing different superscripts within a column differ significantly. * $P<0.05$, ** $P<0.01$, NS-Non-significant.

Table 8: Analysis of variance for cumulative weekly feed conversion ratio of broilers fed with Peppermint Essential oil and different organic acids

Source	df	1 st week		2 nd week		3 rd week	
		MSS	'F' value	MSS	'F' value	MSS	'F' value
Treatments	7	0.005	0.67	0.002	0.93	0.009	12.23
Error	16	0.007	-	0.002	-	0.001	-
		4 th week		5 th week		6 th week	
		MSS	'F' value	MSS	'F' value	MSS	'F' value
Treatments	7	0.012	4.33	0.011	5.86	0.013	3.67
Error	16	0.003	-	0.002	-	0.003	-

MSS - Mean sum of squares, df - Degrees of freedom, CD-Critical difference, CV-Coefficient of variance.

These results are also in agreement with Adil *et al.* [24] who observed that birds fed with diet supplemented with organic acids showed a significant improvement in the FCR as compared to birds fed the control diet. Saki *et al.* [25] reported that inclusion of 0.5 and 1% of organic acids mixture in broiler diet resulted in better feed conversion ratio. Our findings are in agreement with Jamroz *et al.*, [26]; Zhang *et al.*, [27], who have found that supplementing broiler diets with essential oils improves FCR. Moreover, Wade *et al.* [28] observed that the feed supplemented with thyme essential oil at 100mg/kg resulted in improved feed conversion ratio. However, Pappas *et al.* [21] reported that addition of essential oil blend (EO) and benzoic acid in broilers did not significantly affect feed conversion ratio in chickens.

Mortality

The mortality showed normal pattern and was within a normal range in all treatment groups. The mortality from groups A to H was 3.33, 1.67, 3.33, 1.67, 1.67, 3.33, 3.33 and 1.67%, respectively (Table 9).

Table 9: Mortality (%) up to 6th week of age of broilers fed with Peppermint Essential oil and different organic acids.

Treatment groups	No. of birds/treatment	No. of birds died/treatment	Mortality (%)
A	60	2	3.33
B	60	1	1.67
C	60	2	3.33
D	60	1	1.67
E	60	1	1.67
F	60	2	3.33
G	60	2	3.33
H	60	1	1.67

The dietary treatments did not found to influence the mortality in broilers under different treatment groups. Saki *et al.* [25] reported that inclusion of 0.5 and 1% of organic acids mixture organic acid in broiler diet has no effect on mortality in this respect. Wade *et al.* observed improved FCR with supplementation of thyme essential oil. Pappas *et al.* [21] found that addition of essential oil blend (EO) and benzoic acid did not significantly affect mortality in broilers.

Total viable count and *E. coli* count

The statistical analysis revealed that the total viable count was reduced significantly ($P<0.01$) in all treatment groups compared control (Table 10). Treatment group E showed numerically lower total viable count among all treatment groups but the difference was statistically non-significant. The *E. coli* count was significantly ($P<0.01$) reduced in treatment group E, F, G and H offered blend of organic acid and essential oil compared to control and other treatment groups.

Table 10: Bacterial count log₁₀ (CFU/gm) of broiler chicken intestine at 21st day fed with Peppermint Essential oil and different organic acids.

Treatment group	TVC log ₁₀ (CFU/gm)	Coliform log ₁₀ (CFU/gm)
A	8.28 ± 0.01 ^a	7.26 ± 0.02 ^a
B	7.78 ± 0.02 ^b	6.81 ± 0.13 ^b
C	7.79 ± 0.11 ^b	6.77 ± 0.04 ^b
D	7.84 ± 0.11 ^b	6.74 ± 0.02 ^b
E	7.78 ± 0.08 ^b	6.66 ± 0.11 ^{bc}
F	7.64 ± 0.07 ^b	6.56 ± 0.04 ^{bc}
G	7.69 ± 0.06 ^b	6.60 ± 0.07 ^{bc}
H	7.76 ± 0.08 ^b	6.43 ± 0.17 ^c
CD	0.314**	0.379**
	0.228 *	0.275 *
CV%	1.683	2.363

Means bearing different superscripts within a column differ significantly. * $P<0.05$, ** $P<0.01$,

Table 11: Analysis of variance for Bacterial count log₁₀ (CFU/gm) of Broiler Chicken Intestine at 21st day fed with Peppermint essential oil and different organic acids.

Source	Df	Total viable count		Coliform count	
		MSS	'F' value	MSS	'F' value
Treatments	7	0.115	6.64**	0.185	7.30**
Error	16	0.017	-	0.025	-

MSS - Mean sum of squares, df - Degrees of freedom, CD-Critical difference, CV-Coefficient of variance.

Jamroz *et al.* [26] reported the positive impact on gut microbiota with capsaicin, carvacrol and cinnamaldehyde supplementation reduced the CFU of *Escherichia coli* to a limited extent. Akyurek *et al.* [29] reported that broiler chickens fed diets containing organic acid blends had less pathogenic bacterial load such as coliforms and Clostridia. Thymol and cinnamaldehyde blend showed a synergistic effect in promoting a healthy gut microflora and reducing potentially harmful bacteria [23]. Aksu and Bozkurt [30] observed that a blend of essential oils with or without humic acids decreased *E. coli* count and increased Lactobacilli in intestinal microflora. Roofchae *et al.*, [31] found that populations of cecal *Escherichia coli* were significantly lower in 300 and 600 mg/kg oregano essential oil supplemented groups in comparison with the control and 1200 mg/kg oregano essential oil supplemented groups.

Conclusions

The results of the present study suggested that dietary supplementation of peppermint essential oil in combination with sorbic acid improved body weight, weight gain and FCR in broilers than their individual effect when administered separately. Moreover, combining acidifier with essential oil may successfully improve performance and gut health in broilers.

References

1. Abudabos AM, Alyemni AH, Dafallah YM, Khan RU. The effect of phy-togenic feed additives to substitute in-feed antibiotics on growth traits and blood biochemical parameters in broiler chicks challenged with *Salmonella typhimurium*. Environmental Science Poll Res 2016; 23:24151-24157.
2. Abudabos AM, Alyemni AH, Dafalla YM, Khan RU. Effect of organic acid blend and *Bacillus subtilis* alone or in combination on growth traits, blood biochemical and antioxidant status in broiler exposed to *Salmonella typhimurium* challenge during the starter phase. Journal of Applied Animal Research. 2017; 45:538-542.
3. Tehseen M, Tahir M, Khan RU, Jabbar A, Ahmad B, Ahsan T, et al. Additive Effect of *Nigella sativa* and *Zingiber officinale* Herbal Mixture on Performance and Cholesterol Profile in Broilers. The Philippine Agricultural Scientists. 2016; 99:408-413.
4. Weber GM, Michalczuk, Huyghebaert G, Juin H, Kwakernaak C, Gracia MI. Effects of a blend of essential oil compounds and benzoic acid on performance of broiler chickens as revealed by a meta-analysis of 4 growth trial in various locations. Poultry Science. 2012; 91:2820-2828.
5. Giannenas I, Florou P, Papazahariadou M, Cheristaki E, Bostoglou NA, Spais AB. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. British Poultry Science. 2003; 57:99-106.
6. Janatan IB, Yassin MS, Chin CB, Chen LL, Sim NL. Antifungal activity of the essential oils nine zingiberaceae species. British Poultry science. 2003; 41:392-397.
7. Bostoglou NA, Florou-paneri EC, Giannenas I, Papageorgiou G, Spais AB. The effect of a mixture of herbal essential oils or α -tocopheryl acetate on performance parameters and oxidation of body lipid in broilers. South African Journal Animal Science. 2004; 34:52-61.
8. Pirgozliev V, Murphy T, Owens, George BJ, McCann ME. Fumaric and sorbic acid as additives in broiler feed. Research in Veterinary Science Livestock Research for Rural Development. 2008; 84(3):387-394.
9. Ao T, Cantor AH, Pescatore AJ, Ford MJ, Pierce JL, Dawson KA. Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. Poultry science. 2009; 88:111-117.
10. BIS Bureau of Indian Standards, Poultry Feeds Specification (5th Revision). IS: 1374-Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi-11., 2007,
11. DIFCO Manual of microbiological culture media. 1977; 2:646-647.
12. ISO, Recommendation of the meeting of the subcommittee, International Organization for Standardization, on meat and meat products. ISO/TC-36/Sc-6. The Netherlands. 1995, 10-18.
13. Snedecor GW, Cochran WG. Statistical Methods. 8th Edn. IOWA State University Press, Ames, IOWA, US, 1994.
14. Al-Kassie GAM. Influence of two plant extracts derived from thyme and cinnamon on broiler performance. Pakistan Veterinary Journal. 2009; 29(4):169-173.
15. Panda AK, Rao SVR, Raju MVLN, Sunder GS. Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. Asian-Australas Journal of Animal Science. 2009; 22:1026-1031.
16. Alcicek A, Bozkurt M, Cabuk M. The effect of a mixture of herbal essential oils, an organic acid or a probiotic on broiler performance. South African Journal of Animal Science. 2004; 34(4):217-222.
17. Athina Tzora, Ilias Giannenas, Achilleas Karamoutsios, Nikolaos Papaioannou et al. Effects of Oregano, Attapulgit, Benzoic Acid and their Blend on Chicken Performance, Intestinal Microbiology and Intestinal Morphology, Journal of Poultry Science. 2017; 54:218-227.
18. Hassan HM, Mohamed, Youssef AW, Hassan ER. Effect of using organic acids to substitute antibiotic growth promoters on performance and intestinal microflora of broilers. Asian-Aust. Journal of Animal Science. 2010; 23:1348-1353.
19. Alaeldein M, Abudabos, Alyemni AH. Effects of the essential oil blend CRINA® Poultry ill feed on broiler performance and gut microbiology. Italian Journal of Animal Science. 2013; 12(4):234-238.
20. Hernandez F, Garcia, Madrid J, Orenge J, Catala P, Megias MD. Effect of formic acid on performance, digestibility, intestinal histomorphology and plasma metabolite of broiler chickens. British Poultry Science. 2006; 47(1):50-56.
21. Pappas AC, Mountzouris KC, Fegeros K, Zervas G. Effects of essential oils and benzoic acid on broiler performance and gut microflora. Epitheorese Zootechnikes Epistemes. 2011; 41:55-64.
22. Dibner JJ, Buttin P. Use of organic acid as a model to study the impact of gut microflora on nutrition and metabolism. Journal of Applied Poultry Research. 2002; 11:453-463.
23. Bento MHL, Ouwehand AC, Tiihonen K, Lahtinen S, Nurminen P, et al. Essential oils and their use in animal feeds for monogastric animals- Effects on feed quality, gut microbiota, growth performance and food safety: a review. Veterinary medicine. 2013; 58(9):449-458.
24. Adil S, Banday MT, Bhat GA, Qureshi SD, Wani SA. Effect of supplemental organic acids on growth performance and gut microbial population of broiler chicken. Livestock Research for Rural Development. 2011, 23.
25. Saki AA, Eftekhari SM, Zamani P, Aliarabi H, Abbasinezhad. Effects of an organic acid mixture and methionine supplements on intestinal morphology, protein and nucleic acids content, microbial population and performance of broiler chickens. Animal Production Science. 2011; 51(11):1025-1033.
26. Jamroz D, Williczkiewicz A, Wiertelcki T, Orda J, Skorupinska J. Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. British Poultry science. 2005; (46):485-493.
27. Zhang KY, Yan F, Keen CA, Waldroup PW. Evaluation of Microencapsulated Essential Oils and Organic Acids in Diets for Broiler Chickens. International Journal of Poultry Science. 2005; 4(9):612-619.
28. Wade MR, Manwar SJ, Kuralkar SV, Waghmare SP, Ingle VC, Hajare SW. Effect of thyme essential oil on performance of broiler chicken. Journal of Entomology

- and Zoology Studies. 2018; 6(3):25-28.
29. Akyurek H, Ozduven ML, Okur AA, KocF, Samli HE. The effect of supplementing an organic acid blend and/or microbial phytase to a corn-soybean based diet fed to broiler chickens. African Journal Agriculture Research. 2011; 6:642-649.
 30. Aksu T, Bozkurt AS. Effect of dietary essential oils and/or humic acids on broiler performance, microbial population of intestinal content and antibody titers in the summer season. Kafkas University of Veterinary Medicine Journal. 2009; 15(2):185-190.
 31. Roofchae A, Irani M, Ebrahimzadeh MA, Akbari MR. Effect of dietary oregano (*Origanum vulgare* L.) essential oil on growth performance, cecal microflora and serum antioxidant activity of broiler chickens. African Journal of Biotechnology. 2011; 10(32):6177-6183.