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The effects of thyme oil and black cumin oil in broiler feeding on growth performance, intestinal histomorphology, and cecal volatile fatty acids

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Abstract: The aim of the study was to investigate the effects of thyme oil and black cumin oil that are added to broiler rations at certain ratios, on performance (body weight, body weight gain, feed intake, feed conversion ratio, mortality, European Production Efficiency Factor (EPEF), histomorphological parameters of jejunum and ileum, and cecal short-chain fatty acid (SCFA) concentrations. In the study, 216 male chicks (Ross 308) were randomly divided into 4 groups of 54 chicks each. Each group was divided into 9 subgroups of 6 chicks. The chicks were fed with corn, soya bean, and full-fat soybean-based rations for 42 days. While the control group was fed with basic ration, the experimental groups were fed respectively with 250 mg/kg thyme oil (T), 100 mg/kg black cumin oil (B), 250 mg/kg thyme oil, and 100 mg/kg black cumin oil (BT) added to the basic ration. There was no statistically significant difference between the experimental and control groups in terms of performance, mortality, and EPEF. No statistically significant difference was found in all groups in terms of jejunum histomorphology (villus height (VH), crypt depth (CD), and VH/CD ratio). When the ileum histomorphology was examined, there was a statistically significant difference between the control and study groups in terms of crypt depth ($P < 0.05$). On the 42nd day of the study, there was no statistically significant difference between the groups regarding acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid, caproic acid, total SCFA and BCFA (μmol/g) in the cecal digesta. In conclusion, the use of thyme oil and black cumin oil in broiler rations did not affect performance parameters and cecum essential fatty acids, but they had positive effects on intestinal health.

Key words: Intestinal histomorphology, broiler, black cumin, thyme, short-chain fatty acid

1. Introduction

The European Union prohibited the use of antibiotics as a growth factor in animal diets, on 1 January 2006, (Directive 70/524 / EEC and Directive 1831/2003 / EC) [1]. After the definitive ban on antibiotics and other growth factors, a search for new food additives started. During this search, herbal extracts have drawn attention, as they are natural and safe and they have antimycotic, antibacterial, antiviral, antioxidant, and antilipidemic properties [2].

Thyme is a common term for the plant family Lamiaceae, which has more than 60 species and are known by their general aroma and taste [3]. Thyme is rich in carvacrol and at a lesser extent in phenolic monoterpenoids (particularly thymol) [4]. In recent years, they have attracted the attention of the consumers due to their antimicrobial, antimycotic, insecticidal, and antioxidative effects in the human body [5,6]. The black cumin belongs to the subspecies *Nigella sative* of the family Ranunculaceae, which has 14 subspecies. The seeds of the black cumin contain volatile oils, nigellone, carvacrol, p-cymene, d-limonene, α- and β-pinene in addition to other active components. The most important of these components are thymoquinone, dithymoquinone, thymohydroquinone, and thymol [7]. It was reported that thymoquinone is an effective antioxidant [8], anticarcinogenic, and antimutagenic [9, 10].

In recent years, many studies focusing on the aromatic plants and their extracts have been conducted. However, in the literature review, the number of studies evaluating the intestinal histomorphology of essential oils of oregano and black seed added to the chick diets was rather limited, and no studies were conducted to investigate the effect of the relationship between volatile fatty acids of cecum.

In this study, our objective was to investigate the effects of thyme oil and black cumin oil, which were added to the broiler rations, on the growth performance, intestinal histomorphology, and cecal volatile fatty acid concentrations.

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2. Materials and methods

2.1. Birds and management

This study was approved by the Local Ethics Committee for Animal Experiments at Ankara University (Approval No: AU-HAYDEK/2014-16-98). In this study 216 one-day-old male chicks (Ross 308) were included and they were divided into 4 groups (54 chicks in each group) and these groups were divided into 9 subgroups (6 chicks in each group). All animals in the control and experimental groups were fed in three periods: In each group, starter feed between 0th and 10th days (23.00% CP and 3000 kcal/kg metabolizable energy (ME)), grower feed between 11th and 24th days (21.50 %CP and 3100 kcal/kg ME) and finisher feed between 25th and 42nd days (19.50% CP and 3200 kcal/kg ME) are given to the broiler (Table 1). The animals were fed with rations based on corn and soy pulp as ad libitum during the trial. The ration components were prepared according to the Aviagen [11]. All animals were given fresh water during the study. The study duration was 42 days. While the control group was fed with the basic ration, the study groups were fed, in addition to the basic ration, with 250 mg/kg thyme oil (T), 100 mg/kg black cumin oil (B), 250 mg/kg thyme oil and 100 mg/kg black cumin oil (BT) respectively. The essential fatty acid profile of thyme and black cumin seed oil used in the study is given in Tables 2 and 3. Thyme oil distillation method and black seed oil were obtained by cold press method. In addition, these plant oils were obtained from the commercial company Botalife (Isparta, Turkey). The body weight of the animals was measured on the 0th, 10th, 24th, and 42nd days. The body weight gain was determined with the comparison of these measurements. Futhermore, feed intake was recorded for each pen, and feed/gain ratio (F/G) was calculated during the same periods to determine the growth performance of birds. On the 42nd day of the study, all the animals were weighed and one broiler from each subgroup was randomly chosen. A total 36 animals were sacrificed with a suitable method for the histomorphological examination, volatile fatty acid analysis and for the determination of the carcass parameters.

The mortality was calculated with the following formula after each period:

Mortality, % = (dead chick number / chick number at start) × 100

The European Production Efficiency Factor (EPEF) was calculated with the following formula at the end of the study:

EPEF = [Body weight (kg) × Viability (%)] / (Age × Feed Conversion Ratio) × 100

2.2. Histomorphological analysis of the intestine

At the end of the study, one broiler from each subgroup (a total of 9 chicks from each group) was sacrificed with a

Table 1. The composition of the rations used in the study (%)[1].

Periods	Starter	Grower	Finisher
Raw material, %	0th–10th days	11th–24th days	25th–42nd days
Corn	40	35	40
Wheat	0	13.85	11
Broken rice	9.3	5	5
Soybean meal	23.42	22.5	20
Soybean (Full fat)	15	10	9
Meat-bone powder	2.75	3	2.5
Chicken powder	2.5	2	2
Vegetable oil	3.5	5.5	7.5
Marble powder	0.55	0.55	0.55
DCP	1.2	1	1
Yeast	0.3	0.3	0.3
DL-methionine	0.35	0.3	0.15
L-lysine HCl	0.15	0.05	0.05
Sodium bicarbonate	0.2	0.2	0.2
Salt	0.3	0.3	0.3
Vit-min mix	0.3	0.3	0.3
Phytase	0.1	0.1	0.1
L-Threonine	0.08	0.05	0.05
Total	100	100	100
The calculated value			
Crude protein, %	23.4	21.6	19.9
ME (kcal/kg)	3064	3126	3274
Lysine, %	1.44	1.26	1.15
Methionine + Cysteine,%	1.09	0.99	0.8
Ca, %	1.04	1.01	0.93
Total P, %	0.51	0.49	0.45
Analysis values:			
kcal/kg	3045.46	3140.70	3259.92
CP %	23.18	21.40	20.73

[1]As-fed basis.

[2]Provided per kilogram of complete diet: vitamin A, 12,000 IU; vitamin D3, 2500 IU; vitamin E, 40 IU; vitamin K3, 5 mg; thiamin, 2.5 mg; riboflavin, 6 mg; pyridoxine, 5 mg; pantothenic acid, 15 mg; niacin, 25 mg; folic acid, 1 mg; biotin, 50 µg; vitamin B12, 20 µg.

[3]Provided per kilogram of complete diet: Cu, 5 mg; I, 1 mg, Co, 200 µg; Se, 150 µg; Fe, 60 mg; Zn, 60 mg; Mn, 80 mg. Folic Acid 1000 mg/kg, Biotin 50 mg/kg, Copper 5000 IU/kg, Iodine 1000 IU/kg, Cobalt 200 mg/kg, Selenium 150 mg/kg, Iron 60,000 mg/kg, Zinc 60,000 mg/kg, Mangan 80,000 mg/kg.

Table 2. The essential oil acid profile of thyme oil (%).

Ingredient	%	Ingredient	%
α-pinene	0.29	*spathulenol*	0.37
α -thujene	0.30	*b-caryophyllene oxide*	1.01
α- terpinene	0.66	*carvone*	0.25
b-myrcene	0.79	*α -terpineol*	0.32
α -fellanderen	0.30	*borneol*	0.99
g-terpinen	2.39	*b-bisabolen*	2.08
p-cymene	2.89	*timol*	4.15
Linalyl acetate	0.35	*carvacrol*	75.30
linalool	6.36		

Table 3. The essential oil acid profile of black cumin oil (%).

Ingredient	%	Ingredient	%
α-pinene	0.91	*Longifenole*	5.53
α -thujene	4.10	*α -longipinene*	1.97
g- terpinene	0.58	*carvacrol*	0.38
b-piene	1.47	*thymoquinone*	50.11
limoene	1.63	*Terpinen-4-ol*	0.81
terpiolene	0.55	*b- cyclocitral*	0.59
p-cymene	29.66		

suitable method and their intestines were quickly separated from the carcass. In order to provide uniformity for each animal, specimens were obtained from 8 cm proximal of Meckel's diverticula for jejunum and from 8 cm proximal of the ileocecal junction for ileum. Each intestinal specimen (jejunum and ileum) had a size of 1 cm. Tissue samples were fixed in 10% neutral buffered formaline for 24 h and washed with tap water; they were subsequently dehydrated in graded ethanol solutions, cleared with xylol, and embedded in paraffin, respectively. Intestinal segments were sectioned at the thickness of 5 µm with microtome. Cross-sections were prepared and stained with Mallory's triple stain modified by Crossman in order to determine the intestinal morphometry. Villus height was measured from the top of the villus to the crypt mouth, and crypt depth was defined as the depth of the invagination between adjacent crypt mouths. Villus width was measured at the bottom of the villus (Figure 1). Histological sections were examined under light microscope (Leica DM 2500, Leica Microsystems GmbH, Wetzlar, Germany) and photographed with Leica DFC450 (Leica Microsystems, Heerbrug, Germany) digital microscope camera. The images were evaluated using ImageJ software (Image J, US National Institutes of Health, Bethesda, MD, USA) [12].

2.3. The concentrations of some volatile fatty acids in cecum

The cecal digesta obtained after the sacrifice of the animals was used for the determination of acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, caproic acid, and isocaproic acid with a gas chromatography (GC) device (Shimadzu GC, Shimadzu Co., Kyoto, Japan) and colons (Teknokroma; TR-151035,

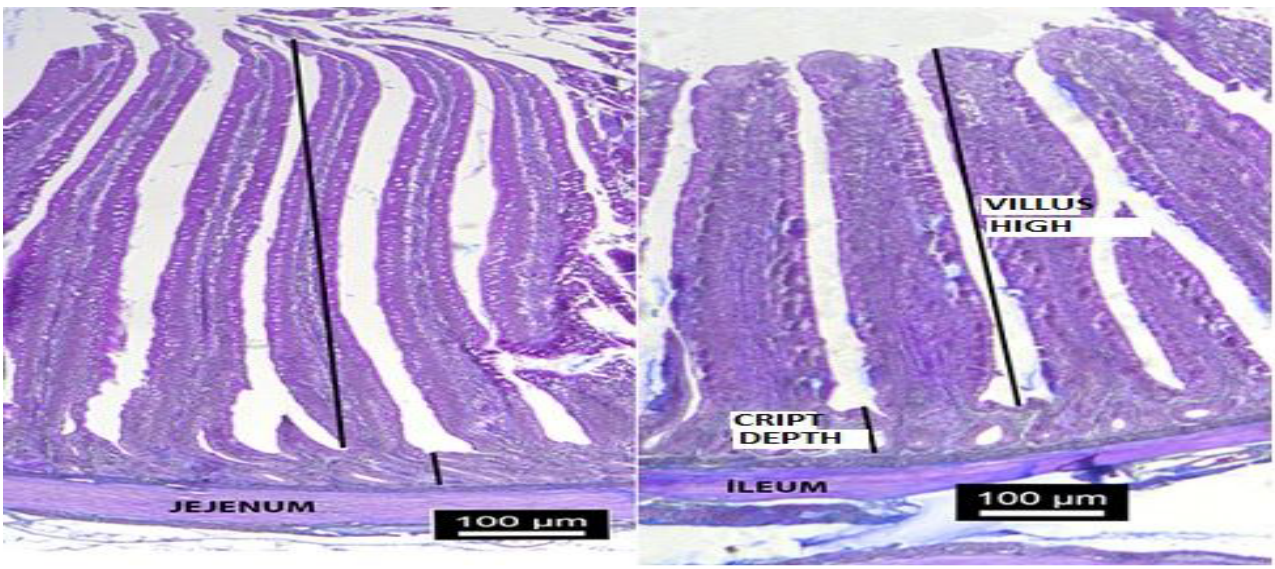

Figure 1. Histomorphological measurements of jejunum and ileum. Villus height and crypt depth. Crossman's triple staining technique e. Bar: 100 µm.

TRB-FFAP 30m × 0.53 mm × 0.50 µm). Helium was used for the carrier gas and the colon temperature was programmed so that it was increased stepwise from 120 °C to 160 °C. In addition, the FID (Flame Ionization Detector) and injector block temperature was set to 250 °C and 230 °C respectively. The analysis was performed according to the protocol described by Zhang et al. [13]. The calibration curve was drawn with the Supelco Volatile Free Acid Mix, 46975-U (10 mmol/L) as a standard (Figure 2).

2.4. Statistics analysis

The one-way variance analysis method (ANOVA) was used for the statistical calculations of the groups and the importance of the differences between the mean values in the groups and a suitable post hoc test (Tukey) was used for the importance control of the differences between the groups. Chi-square test was used for the determination of the difference between the groups regarding the mortality rate. The statistical analysis was done with the SPSS software package [14].

3. Results

There was no statistically significant difference between the control group and the study groups (T, B, BT) regarding the mean body weight gain, mean feed intake, feed conversion rate, mortality, and EPEF (Table 4).

The morphological measurements of ileum and jejunum were shown in Table 5. There was no statistically significant difference between the control group and the study groups regarding villus height (VH), crypt depth (CD), and VH/CD ratio after the addition of different plants and aromatic plant extracts with different doses. Ileum: Although, there was no statistically significant difference in villus height and VH/CD ratio between the control group and the study groups, the difference between the groups in terms of crypt depth was statistically significant ($P < 0.05$). We observed that the addition of black cumin oil to the ration increased significantly the crypt depth in the study groups compared to the control group. In groups, in which plant extract was added to the ration, the crypt depth was higher than that in the control group. We determined that the crypt depth was affected by the addition of the plant oil to the ration.

The SCFA and BCFA concentrations in the cecal digesta (µmol/g) are summarized in Table 6. The levels of acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid, caproic acid, total SCFA and BCFA in the cecal digesta in the control group and the study groups on the 42nd day of the study are shown in Table 6. There was no statistically significant difference between the groups.

4. Discussion

The plants show their effects through the active compounds like carvacrol, thymol, linalool, cineol and with the functional and structural damage caused usually on the plasma membranes of the pathogenic microorganisms [15]. Carvacrol and thymol destroy the bacterial membrane and cause the release of the substances of the membrane to the extracellular medium. The terpenoids and phenylpropanoids can penetrate the bacterial membrane thanks to their lipophilic properties and can access more inner parts of the cells [16]. Further studies are needed to demonstrate the effects of the volatile fatty acids in thyme and black cumin oils on the performance and bowel health depending on their effective doses. In recent studies, in which 100 and 200 ppm thyme essential oil [17] 1.5%, 2%, 2.5%, and 3% black cumin seeds [18] and essential oil mixture containing black cumin seeds and thyme leaves [19,20] were used, it was reported that they had positive effects on body weight gain. However, in some studies, in which 50 and 100 mg/kg thyme oil [21],

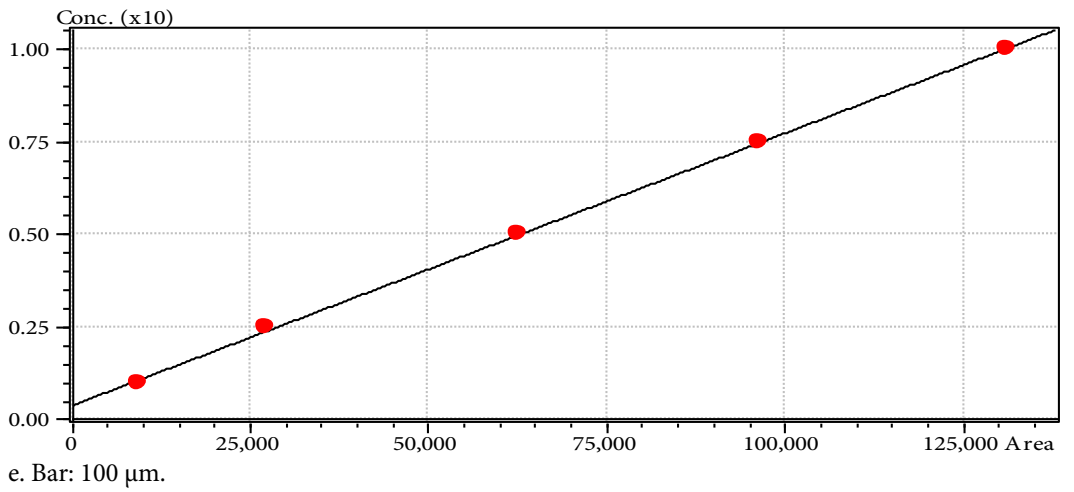

Figure 2. The volatile fatty acid calibration curve.

Table 4. Effects of dietary supplementation of thyme and black cumin oil on body weight gain, feed intake, feed conversion ratio, mortality, and EPEF in the broiler chickens.

	Dietary treatment								
Item	Control		T		B		BT		
0th–10th days	$\bar{x}$	S $\bar{x}$	$\bar{x}$	S x$\bar{x}$	$\bar{x}$	S $\bar{x}$	$\bar{x}$	S $\bar{x}$	P
BWG (g)	277.72	10.11	274.33	3.75	283.20	4.58	271.54	6.95	0.657
FI (g)	340.55	10.32	347.37	8.42	349.40	5.65	333.92	9.03	0.571
FCR	1.23	0.01	1.26	0.02	1.23	0.01	1.23	0.01	0.474
11th–24th days									
BWG (g)	1064.50	10.55	1035.46	12.36	1033.58	22.64	1043.11	17.36	0.533
FI (g)	1339.05	50.25	1385.07	37.00	1404.97	24.96	1427.59	27.34	0.373
FCR	1.28	0.03	1.33	0.03	1.36	0.01	1.36	0.01	0.134
25th–42nd days									
BWG (g)	1750.36	34.41	1844.94	53.22	1781.57	21.17	1800.26	23.24	0.308
FI (g)	3044.49	51.28	3149.00	72.16	3142.46	71.10	3031.40	41.09	0.377
FCR	1.74	1.68	1.71	1.65	1.76	1.66	1.68	1.62	0.286
0th–24th days									
BWG (g)	1342.23	9.24	1309.79	14.01	1316.78	25.15	1314.65	22.48	0.622
FI (g)	1679.61	58.36	1732.44	40.59	1754.38	28.17	1761.51	35.44	0.518
FCR	1.26	1.18	1.32	1.25	1.33	1.30	1.34	1.30	0.122
0th–42nd days									
BWG (g)	3092.60	29.49	3154.74	57.26	3098.36	30.77	3114.91	32.93	0.679
FI (g)	4724.10	60.34	4881.45	79.65	4896.84	62.66	4792.92	67.92	0.259
FCR	1.53	1.48	1.55	1.50	1.58	1.51	1.53	1.50	0.365
EPEF	454.36		466.66		450.79		482.37		0.349
Mortality, %	5.6		3.7		3.7		0		0.423

Mean ($\bar{x}$) and standard error (S$\bar{x}$) values of 9 subgroups in each group.
C: Control group, T: 250 mg/kg thyme oil, B: 100 mg/kg black cumin oil, BT: 250 mg/kg thyme oil and 100 mg/kg black cumin oil.
BWG = body weight gain; BW = body weight; FI = feed intake; FCR = feed conversion ratio.
EPEF = European Production Efficiency Factor [Viability (%) × body weight (kg) × age (d)/FCR× 100].

2–4 g/kg black cumin [22], thymol, cinnamaldehyde, and essential oil mixture [23] were used, it was reported that these feed additives did not have any effect on the body weight gain. In other studies, in which thyme leaves (70 mg/kg) [24], commercial essential oil mixture of several aromatic plants including thyme [19] were used, it was reported that these feed additives did not affect feed consumption. However, it was reported in some studies that the addition of the plant extracts to the broiler rations decreased the feed consumption [25]. In the studies, in which broiler rations enriched with 5 g/kg and 7.5 g/kg thyme [26], black cumin [27], and commercial essential oil mixtures were used, it was reported that they did not affect the feed conversion ratio. On the other hand, there are also some studies, which reported that feed conversion ratio was improved due the addition of thyme, black cumin, or commercial essential mixtures [28,29]. The thyme and black cumin oil, which we had used in our study, did not cause any adverse effect on the broiler performance.

In the studies in which aromatic plants and extracts that are added as food additives to the broiler rations mortality rates did not significantly change within the 0–42-day feeding period [19]. This result was consistent with that of our study. Although it seems that the addition of the thyme and black cumin in the rations did not significantly affect the mortality rate, we observed a positive effect in our study. In the group in which thyme and black cumin oil were added to the rations, we did not encounter any

Table 5. The effect of the thyme and black cumin oils in the ration on the jejunum and ileum histomorphology (μm).

Groups	n	C		T		B		BT		P
	9	x̄	S x̄	x̄	S x̄	x̄	S x̄	x̄	S x̄	
Jejenum villus height	9	1033.7	54.98	1050.3	62.0	1068.9	57.26	1036.2	38.83	0.965
Jejenum crypt depth	9	183.04	11.40	171.8	11.5	191.72	8.37	203.1	10.99	0.221
Jejenum villus height/crypt depth		5.76	0.39	6.46	0.73	5.63	0.33	5.23	0.37	0.361
Ileum villus height	9	762.78	39.33	851.37	26.4	852.06	48.4	900.01	29.6	0.086
Ileum crypt depth*	9	136.46^{b}	5.46	148.87^{ab}	6.03	168.12^{a}	8.72	156.86^{ab}	7.12	0.022
Ileum villus height/crypt depth	9	5.67	0.38	5.81	0.33	5.17	0.40	5.86	0.38	0.567

The mean (x̄) and standard error (Sx̄) values of 9 subgroups in each group. C: Control group, T: 250 mg/kg thyme oil, B: 100 mg/kg black cumin oil, BT: 250 mg/kg thyme extract and 100 mg/kg black cumin oil. *a,b; The differences between the mean values with a different letter in the same row were statistically significant (P < 0.05).

Table 6. The effect of the addition of thyme and black cumin oils to rations on some cecal volatile fatty acid concentrations (μmol/g).

Groups	n	C		T		B		BT		P
	9	x̄	S x̄	x̄	S x̄	x̄	S x̄	x̄	S x̄	
Acetic acid	9	43.18	3.72	39.81	3.03	35.44	2.63	35.74	26.98	0.320
Propionic acid	9	6.77	0.54	5.72	0.20	5.74	0.60	5.82	0.46	0.352
Butyric acid	9	1.70	0.05	1.75	0.49	1.74	0.05	1.72	0.04	0.935
Butyric acid	9	10.35	1.24	9.19	1.07	8.70	0.89	8.64	1.01	0.651
Isovaleric Acid	9	1.71	0.06	1.77	0.07	1.78	0.07	1.75	0.04	0.888
Valeric Acid	9	2.87	0.20	2.65	0.08	2.94	0.25	2.73	0.15	0.694
Isocaproic Acid	9	1.94	0.18	1.78	0.02	2.28	1.65	1.88	1.59	0.232
Caproic Acid	9	2.16	0.21	1.98	0.02	2.58	0.33	2.09	0.15	0.224
Total SCFA	9	70.74	5.23	64.69	4.22	63.45	5.37	60.41	5.16	0.533
BFCA	9	6.30	0.32	6.19	0.15	6.46	0.36	6.21	0.23	0.896

C:Control group, T: 250 mg/kg thyme oil, B: 100 mg/kg black cumin oil, BT: 250 mg/kg thyme extract and 100 mg/kg black cumin oil. Statistically not significant (P >0.05). The mean (x̄) and standard error (Sx̄) values of 9 subgroups in each group.
BCFA (branched chain fatty acids): isobutyric acid + isovaleric acid + valeric acid.
Total SCFA: acetic acid + isobutyric acid + butyric acid + isovaleric acid + valeric acid.

death depending on the synergistic effect. The European Production Efficiency Factor (EPEF) is a performance parameter that is referred by the breeders to determine the herd performance in the integrated facilities. Studies showed that the highest EPEF score was found in the groups, which were fed with the aromatic plants and extracts [30]. This result was consistent with our study.

Morphological changes in the small intestine, villus height, villus width, and villus height crypt depth ratio (VH/CD) may improve poultry performance by improving nutrient digestion and absorption [31]. Ghazanfari et al. [32] indicated that the mucosal morphology of the jejunum was not affected by the feed additive aromatic plants and extracts. The result is consistent with that of our study. However, in the study conducted by Boka et al. [33], the researchers used 0%, 1%, 2%, and 3% black cumin in the laying hens and reported that 2% black cumin increased significantly the villus height, crypt depth, and VH/CD parameters in the jejunum. Catalá et al. [34] reported that the herbal extract mixture consisting of carvacrol, cinnamaldehyde, and capsaicin and the antibiotics had a positive effect on the ileal morphology,

and villus height and villus surface areas were significantly increased in the groups fed with herbal extracts. In a study, in which *Camellia oleifera* seeds were used, villus height, crypt depth, and VH:CD values were significantly increased and the barrier function of the bowels was improved [32,35]. In another study, broilers were fed with aromatic plants and extracts and a decrease in the jejunal crypt depth was observed [36]. Murugesan et al. [29] used commercial aromatic plants and extracts as food additives and reported that the ileal villus height was increased but crypt depth was decreased. Recent studies related to ileum showed that villus height and crypt depth were significantly increased with commercial aromatic plants and extracts used as food additives [37,38]. Hashemipour et al. [39] added 0, 100, and 200 mg/kg carvacrol + thymol mixture to the rations and showed that villus surface, VH/CD ratio, crypt depth, and muscular layer were built up in jejunum and ileum in groups fed with 100 and 200 mg/kg. These results are in conflict with our results. Cardoso et al. [36] demonstrated that aromatic plants and extracts did not affect the crypt depth in the ileum in broilers but villus height was increased. In different studies, in which broilers were fed with a plant mixture containing thyme, a conflicting increase in the villus height and VH/CD ratio and a confirming increase in the crypt depth was observed [35,40]. In our study, there was no statistically significant difference between the groups regarding the ileal villus height and VH/CD ratio, but there was a statistically significant difference between the groups for crypt depth.

Short chain fatty acids are formed as a result of bacterial fermentation in the cecum. They stimulate cell growth and differentiation in the intestine, improving intestinal integrity, as well as reducing the digestive tract pH and preventing the growth of pathogenic microorganisms [41]. There are only a limited number of studies focusing on the use of aromatic plants and extracts and on the examination of the parameters related to the concentration of some volatile fatty acids in the cecum. Even though there are a few studies on the relationship between the thyme and black cumin oil usage and the concentration of some volatile fatty acids, no study focusing on only black cumin was encountered. In one study, in which essential oil mixture containing also thyme and organic acid were used in the feeding of male turkeys, there was no statistically significant difference between the groups regarding the cecal SCFA production [42]. This finding was in consistency with our results. However, in a study, in which a mixture of thymol (15 g/t) and cinnamaldehyde (5 g/t) was used, the butyrate rate was increased on the 20th and 41st days and acetic acid rate was decreased on the 20th day and the propionic acid and isovaleric acid rates were decreased on the 41st day [43]. In another study, in which a mixed plant extract (100 g/t) and additive enzymes were used for the feeding of broilers, the rate of the cecal volatile fatty acids did not change on the 22nd day and the cecal acetate and butyrate rates were increased while the propionate rate was decreased in the groups with added extracts [44]. In another study, various plant extracts including thyme oil were used. In the study group, which was fed with the thyme essential oil, woundwort, rosemary, and garlic, the cecal isobutyric acid and isovaleric acid rates were lower than those in the control group [45].

The conflicting results might depend on the herbal factors like the type and dose of the used plant extracts, the volatile fatty acids and active ingredient ratio and interactions and ration composition, coop conditions, and environmental factors.

5. Conclusion

As a result, when the performance parameters of the addition of thyme and black seed oil to broiler rations in terms of mortality, EPEF were not found statistically significant, but significant differences were found for numerical differences. There was no statistically significant difference in intestinal histomorphological parameters between the groups for jejenium (villus height, crypt depth and VH/CD ratio). No statistically significant difference was found between the groups in terms of villus height and VH/CD ratio for the ileum, whereas the difference between the groups in terms of crypt depth was statistically significant ($P < 0.05$). When acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid, caproic acid, and total SCFA and BCFA values were examined, the difference between the groups for all parameters was not statistically significant.

In this study, the positive effects of thyme and black seed oil added to broiler diets on performance parameters remained numerical but positive effects on intestinal health. This can increase profitability for poultry industry. Improvement in intestinal health is very important for herd health. In order to elucidate the mentioned parameters, many studies with different doses are needed in this field. In broiler rations, thyme and black seed oil has been found to be a reliable feed additive. Our study is a good source for further studies.

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Conflict of interest

The authors declare that they have no conflict of interest.

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