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Can thyme (*Thymbra spicata* L. var. *spicata*) volatile oil alleviate the detrimental effects of high stocking densities in Japanese quail?

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Abstract: This study was conducted to observe the effects of thyme plant *(Thymbra spicata* L. var. *spicata)* volatile oil on quails and intestinal microbiota in high stocking density. For this purpose, 300 7-day-old Japanese quails (*Coturnix coturnix Japonica*) were used in the study. The quails were selected on the basis of body weight and divided into 6 groups, each consisting of 5 replications that contained 50 quail chicks according to the following experimental design: positive control group (NSD - CONT), with normal stocking density and no additive; negative control group (HSD - CONT), with high stocking density (HSD) and no additive; group HSD - ANT, with HSD and 10 mg/kg of avilamycin additive, and other groups named HSD - T1, T2, and T3, with HSD and 200, 400, and 600 mg/kg of volatile oil additive respectively. In the study, a stocking density of 160 cm^2/quail was chosen, while the high stocking density (HSD) was 90 cm^2/quail. A statistically significant difference was found in terms of improvements in feed intake and feed conversion ratio ($P < 0.01$), and the highest total bacteria count (cfu/g) was observed in HSD - CONT group ($P < 0.01$). The addition of zahter-thyme volatile oil in the diet of quails kept in high stocking density provided improvements at various levels with regard to intestinal microbiota and animal performance. It was observed that the inclusion of zahter-thyme volatile oil, in particular at 600 mg per kg level, reduced the detrimental effects of stress generated by high stocking density.

Key words: Thyme volatile oil, high stocking density, performance, intestinal microbiota, Japanese quail

1. Introduction

In poultry production, many factors, such as stocking density, temperature, transportation, and vaccination, cause stress to the animals. This situation reduces the rate of profit by decreasing the quantity and quality of the product because stress weakens the immune system and impairs intestinal microflora balance [1,2]. A lot of research has been done on the impact of stocking density, and as a result of the temperature stress in the dense population, there have been found significant changes in growth rate and intestinal pathogens [3]. Poultry are very susceptible to pathogenic bacteria, such as *Escherichia coli*, *Salmonella* spp., *Clostridium perfringens*, and *Campylobacter sputorum*. The regulation of intestinal content has an important role in disease control [4]. Since the 1950s, antibiotics have been largely used as a traditional remedy to treat diseases and control stress factors. They have been supplemented into poultry feeds at around 1% of all treatment doses. Antibiotics that are effective only in the intestines without a systemic effect have been used as feed additives [5]. It has been reported that antibiotics used as a growth factor affect the digestive system by controlling microbial activity, reducing the toxic compounds produced by bacteria, and changing the morphology of the intestinal wall by increasing the absorption of nutrients [6]. As a result of the widespread and long-term use of antibiotics as feed additives and supplements for the purpose of accelerating growth in animals, the risk of developing antibiotic resistant strains has been increased [7]. Therefore, the use of volatile oils as an alternative to antibiotics has emerged. Due to their reliability in terms of chemical structure, harmlessness to human health, ability to stimulate growth, and role in preventing subclinical infections, they have a high potential in poultry farming [8].

Examination of the minimum inhibitory concentration (MIC) of volatile oils with respect to *Escherichia coli* (*E. coli*), *Staphylococcus aureus*, and *Candida albicans (C. albicans)* showed that thyme volatile oil had the most effective value. It was reported that thymol and carvacrol prevented the growth of *Escherichia coli* and decreased the number [9,10].

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The purpose of the present research is to investigate the effects of thyme plant (*Thymbra spicata* L. var. *spicata*) volatile oil, which grows naturally in Turkey's Mediterranean mountainous regions, on quails and intestinal microbiota in high stocking density.

2. Material and methods

2.1. Animals, feeds, and experimental design

This study was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Board (Approval no: 2012/09-03). A total of 300 quail chicks (*Coturnix coturnix japonica*) which were selected with respect to live weight and sex when they were 7 days old were used in this study for 28 days. All the chicks were individually weighed and randomly assigned to treatment groups so that they were homogeneous in terms of sex (6 treatments, 5 replicates, and 50 chicks per group). The birds were housed in cages whose floor spaces measured 50 × 100 cm each. Lighting program was 24 h during the experimental period. Basal diet, which contained 221 g/kg crude protein and 12.6 MJ/kg metabolizable energy, offered ad libitium. The calculated analysis and ingredients of the basal diet are demonstrated in Table 1. Thyme volatile oil was added daily to the basal diet. Water was also given ad libitum with a nipple drinker throughout the experimental period. Experimental groups were designed as follows: the positive control group (NSD-CONT), with normal stocking density (NSD, 160 cm^2/quail) and no additive; the negative control group (HSD – CONT), with high stocking density (HSD, 90 cm^2/quail) and no additive; group HSD – ANT, with HSD and 10 mg/kg of avilamycin; HSD - T_1 group, with HSD and 200 mg/kg of thymbra volatile oil; group HSD - T_2, with the HSD and 400 mg/kg of thymbra volatile oil; and group HSD - T_3, with the HSD and 600 mg/kg of thymbra volatile oil.

Table 1. Composition and contents of nutrients of the basal diet (g/kg).

Ingredients	Composition
Maize	515.0
Wheat	77.0
Wheat bran	45.0
Extracted soybean meal	275.0
Fish meal	55.0
Vegetable oil	15.0
Limestone	10.0
Dicalciumphosphate	7.5
Sodium chloride	2.5
Vitamin-mineral premix*	5.0
Calculated nutrients	
ME, (MJ kg^{-1}) **	12.6
Crude protein (g kg^{-1})	221
Ca (g kg^{-1})	9.0
P (g kg^{-1})	6.0
Lysine (g kg^{-1})	11.0

* Vitamin and mineral premix provides the following per kg: all-trans-retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; all-rac-α-tocopherol acetate, 1.25 mg; menadione (menadione sodium bisulphate), 1.1 mg; thiamine (thiamine mononitrate), 1.1 mg; riboflavin, 4.4 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; pyridoxine, 2.2 mg; folic acid, 0.55 mg; cyanocobalamin, 0.02 mg; Mn, 74 mg (from MnO); Zn, 45 mg (from ZnO); Cu, 4 mg (from CuO); Fe (from FeSO4), 12.5 mg; I (from KI), 0.3 mg; Se (from NaSe), 0.15 mg. ** ME: Metabolizable energy.

2.2. Plant material

The volatile oil was extracted from zahter- *Thymbra spicata* L. var. *spicata* plant, which grows naturally in the province of Hatay, Turkey. Blooming plants were collected from their locations and dried at 35 °C.

2.3. Characterization of volatile oil

Determination of the volatile oil components was carried out with Thermo Scientific ISQ Single Quadrupole model gas chromatograph. TG-Wax MS-A model, 5% Phenyl Polysilphenylene-siloxane, 0.25 mm inner diameter × 30 m length, and 0.25 µm film thickness column were used. Helium (99.9%) was used as the carrier gas at a flow rate of 1 mL/min. The ionization energy was set at 70 eV, and the mass range m/z at 1.2–1200 amu. For collection of data, Scan Mode was used.

The MS transfer line temperature was 250 °C; the MS ionization temperature was 220 °C; the injection port temperature was 220 °C; the column temperature was initially 50 °C, and the temperature was increased up to 220 °C at a rate of 3 °C/min. The structure of each compound was defined using mass spectra by Xcalibur program.

2.4. Determination of performance parameters

The live weights, live weight gains, feed consumption, and feed conversion rates of the quails were determined by the weighing and calculations made every week. On the 35th day of the trial, all the animals were weighed individually, and a total of 120 quails were randomly selected, weighed, and wing-marked for slaughtering. The hot carcass yield was calculated by dividing the slaughtered and eviscerated carcass weight by the preslaughter live weight.

2.5. Determination of microbiology parameters

At the end of the experiment, 120 quails in total (10 females and 10 males from each group), were randomly selected, and then cervical dislocation (from the vena jugularis)

was performed. The content of the small intestine taken from the far end of the duodenum ileo-cecal was kept in ice stored in a glass petri dish until taken to the laboratory for population counting. The samples were evaluated according to CLSI (Clinical and Laboratory Standards Institute) criteria by using conventional methods, and the total bacterial counts were evaluated in terms of the numbers of coliform and lactobacillus bacteria by incubation in aerobic and anaerobic conditions.

2.6. Statistical analysis

SPSS 11.5 package program was used for statistical analysis (SPSS Inc., Chicago, IL, USA). The two-way ANOVA test was used to compare groups. Duncan's multiple comparison test was applied to determine the difference between the groups. When the results were evaluated statistically, the significance was based on $P < 0.05$. The chi-square test was used to determine the death rates and rates of the research groups.

3. Results

The chemical components of *Thymbra spicata* L. var. *spicata* are shown in Table 2. Table 3 shows the live weight of the groups, live weight gain, feed consumption, feed conversion ratio, hot/cold carcass weights, and hot/cold carcass yields. In Table 4, the effect on intestinal microbiota is shown. Thyme oil consists of 24 components, and the main components are carvacrol (71.6%), 0-Cymene (9.03%), and γ-terpinene (5.83%); it also forms a high level of phenolic components including 72% of phenol and 21% of hydrocarbon.

When the live weight and live weight gain of the groups were examined (Table 3), the stocking density started to show negative effects on related parameters from day 14, and the negative control group (HSD - CONT) showed a statistically significant decrease in live weight and live weight gain during all weeks compared to all the other groups. At the end of the study, the negative control group (HSD - CONT) had the lowest live weight (241.52 ± 2.85 g), and the live weight of the positive control group (NSD - CONT) was 290.59 ± 3.02 g ($P < 0.01$). The application of antibiotics and thyme extract at various levels to quails exposed to high stocking density stimulated growth from the 14th day and removed the negative effects of stocking density. It was determined that the stimulant and curative effects in the additive groups were similar ($P > 0.05$) and kept the live weight and live weight gain at the same level as the positive control group (NSD - CONT). The effect of sex on live weight and live weight gain was found to be significant ($P < 0.01$). At the end of the experiment, the female quails' live weight (288.79 ± 2.40 g) was found to be significantly higher than that of males (272.79 ± 2.02 g) ($P < 0.01$). It was determined that the high stocking density suppressed feed consumption, and this effect was more apparent in the latter weeks (Table 3). Indeed, from the 7th to 35th day, the lowest feed consumption was found in the negative control group (691.89 ± 2.23 g), and the highest feed consumption was observed in the positive control group (764.55 ± 2.66 g) ($P < 0.01$). The feed consumption of HSD - ANT group (718.07 ± 1.67 g) was higher than that of the thyme extract groups ($P < 0.01$). In the thyme extract additive groups, i.e. HSD - T2 (695.77 ± 0.68 g) and HSD - T3 (702.61 ± 1.22 g), the feed consumption was significantly lower than it was in HSD - T1 group (706.85 ± 1.48 g) ($P < 0.01$). At the end of the study, it was found that the best performance regarding feed conversion rate occurred in the additive groups ($P < 0.01$), and the stocking density increased feed conversion rate (HSD - CONT, 3.46 ± 0.05). Hot and cold carcass weights were significantly affected by the stocking density, and avilamycin and thyme extract supplemented feed eliminated the suppressive effect of stocking density ($P < 0.01$) (Table 3). The hot and cold carcass weights in the negative control group were

Table 2. Chemical components of *Thymbra spicata* L. volatile oil.

Retention Time (RT)	Rate (%)	Components
18.04	0.67	l-phellandrene
18.62	0.45	δ-3-carene
22.26	0.18	succinaldehyde
22.64	0.77	β -myrcene
24.86	1.06	α-humulene
25.59	9.03	o-cymene
25.83	0.36	cis-D-dihydrocarveol
28.31	5.83	γ-terpinene
29.36	0.26	trans-sabinenehydrate
31.12	0.41	cis-sabinenehydrate
33.99	0.08	3-pinanylamine
34.18	0.93	4-terpineol
36.01	0.31	Z,Z,Z-1,4,6,9-nonadecatetraene
36.93	0.27	thymol
37.12	71.62	carvacrol
39.39	1.91	caryophyllene
39.71	0.18	farnesol
40.01	0.11	trans-Z-alpha-bisaboleneepoxide
40.26	0.44	α-lactose
40.58	0.33	tetra acetyl-d-xlonicnitrile
41.77	4.75	1monolinoleoyglyceroltrimethylsilylether
42.30	0.09	12,15-octadecadienoicacid, methyl ester
42.44	0.53	caryophyllene oxide
47.51	0.42	methylperfluorobutyrate

Table 3. Effect of thyme volatile oil addition in quail rations on performance parameters.

Live weight (g)										
DAY	NSD-CONT	HSD- CONT	HSD-ANT	HSD-T1	HSD-T2	HSD-T3	P	Female	Male	P
7	41.54 ± 0.56	41.56 ± 0.49	41.85 ± 0.49	41.66 ± 0.52	41.95 ± 0.52	41.39 ± 0.53	NS	41.80 ± 0.27	41.50 ± 0.32	NS
14	94.36 ± 1.23^{bc}	92.12 ± 1.11 ^{c}	100.69 ± 1.21 ^{a}	96.71 ± 1.37 ^{b}	92.19 ± 1.21^{c}	91.70 ± 1.31^{c}	**	95.80 ± 0.73	93.31 ± 0.77	*
21	179.36 ± 1.96^{a}	149.61 ± 1.62^{b}	181.48 ± 1.93 ^{a}	181.30 ± 2.08 ^{a}	177.77 ± 1.89^{a}	177.73 ± 2.20^{a}	**	176.69 ± 1.43	172.13 ± 1.43	*
28	259.44 ± 10.2^{a}	203.81 ± 2.32^{b}	243.56 ± 2.35^{a}	253.88 ± 9.55 ^{a}	244.66 ± 2.53^{a}	256.93 ± 9.20^{a}	**	248.97 ± 4.61	238.10 ± 3.88	NS
35	290.59 ± 3.02^{a}	241.52 ± 2.85^{b}	290.33 ± 3.24 ^{a}	287.82 ± 3.50 ^{a}	286.97 ± 2.72^{a}	289.03 ± 3.70^{a}	**	288.79 ± 2.40	272.31 ± 2.02	**
Live weight gain (g)										
7–14	52.81 ± 1.39^{bc}	51.56 ± 1.24^{c}	58.83 ± 1.45 ^{a}	55.04 ± 1.51^{ab}	50.24 ± 1.25^{c}	50.31 ± 1.38^{c}	**	54.00 ± 0.79	51.80 ± 0.86	NS
14–21	84.99 ± 1.13^{ab}	57.49 ± 1.89 ^{c}	80.78 ± 1.12^{b}	84.59 ± 1.78^{ab}	85.58 ± 1.24 ^{a}	86.02 ± 1.46 ^{a}	**	80.88 ± 1.24	78.81 ± 1.09	NS
21–28	69.32 ± 1.20^{a}	54.21 ± 1.23^{d}	62.07 ± 1.16^{c}	63.51 ± 2.05^{bc}	66.88 ± 1.33^{ab}	69.67 ± 1.40^{a}	**	65.65 ± 0.98	62.75 ± 0.83	*
28–35	41.05 ± 1.27 ^{b}	37.71 ± 1.80 ^{b}	46.77 ± 1.57 ^{a}	42.72 ± 1.77^{ab}	41.55 ± 1.11 ^{b}	41.55 ± 2.01 ^{b}	**	45.68 ± 0.93	37.69 ± 0.84	**
7–35	249.04 ± 3.22^{a}	199.97 ± 2.99^{b}	248.47 ± 3.37^{a}	246.29 ± 3.59^{a}	244.99 ± 2.72^{a}	247.40 ± 3.75^{a}	**	246.96 ± 2.44	230.79 ± 2.09	**
Feed Consumption (g)										
7–14	123.04 ± 0.72^{a}	117.04 ± 0.50^{b}	108.24 ± 0.30^{d}	117.23 ± 0.36^{b}	116.05 ± 0.13^{bc}	116.71 ± 0.62^{bc}	**			
14–21	183.57 ± 0.23^{a}	175.91 ± 0.07^{c}	176.10 ± 0.72^{c}	179.33 ± 0.30 ^{b}	166.88 ± 0.14 ^{d}	170.31 ± 0.58 c	**			
21–28	230.14 ± 0.07^{a}	197.32 ± 0.06^{d}	211.03 ± 0.1^{b}	210.85 ± 0.44^{b}	199.97 ± 1.06^{c}	210.40 ± 0.05^{b}	**			
28–35	240.96 ± 2.13^{a}	231.91 ± 2.32^{c}	238.05 ± 1.23^{b}	234.96 ± 1.19^{c}	225.61 ± 1.02^{d}	228.94 ± 1.64^{d}	**			
7–35	764.55 ± 2.66^{a}	691.89 ± 2.23^{d}	718.07 ± 1.67^{b}	706.85 ± 1.48^{c}	695.77 ± 2.72^{d}	702.61 ± 3.70^{c}	**			
Feed conversion ratio										
7–14	2.33 ± 0.05 ^{a}	2.27 ± 0.06 ^{ab}	1.84 ± 0.04^{c}	2.13 ± 0.06^{b}	2.31 ± 0.05 ^{ab}	2.32 ± 0.07 ^{a}	**			
14–21	2.16 ± 0.03^{b}	3.06 ± 0.13^{a}	2.18 ± 0.02^{b}	2.12 ± 0.15^{b}	1.95 ± 0.04^{b}	1.98 ± 0.05^{b}	**			
21–28	3.32 ± 0.05^{b}	3.64 ± 0.09^{a}	3.40 ± 0.07^{b}	3.32 ± 0.10^{b}	2.99 ± 0.06 ^{c}	3.02 ± 0.05^{c}	**			
28–35	5.87 ± 1.09^{ab}	6.15 ± 0.22^{a}	5.09 ± 0.32^{c}	5.50 ± 1.27^{bc}	5.43 ± 0.27^{bc}	5.51 ± 1.26 ^{bc}	**			
7–35	3.07 ± 0.03^{b}	3.46 ± 0.05^{a}	2.89 ± 0.03^{c}	2.87 ± 0.04^{c}	2.84 ± 0.03^{c}	2.84 ± 0.04 ^{c}	**			
Hot-cold carcass weight (g)										
Hot car.	205.54 ± 4.23^{a}	160.89 ± 3.63^{b}	203.93 ± 3.25^{a}	200.57 ± 3.24^{a}	208.23 ± 3.54^{a}	199.16 ± 3.80^{a}	**	197.01 ± 3.67	195.76 ± 3.10	NS
Cold car.	198.95 ± 4.05^{a}	153.57 ± 3.81^{b}	195.43 ± 3.12^{a}	188.80 ± 3.69^{a}	196.60 ± 3.40^{a}	187.63 ± 3.95^{a}	**	186.04 ± 3.62	187.62 ± 2.99	NS
Hot-cold carcass yield (%)										
Hot car.	72.25 ± 0.63^{a}	69.41 ± 0.30^{c}	71.21 ± 0.62^{ab}	71.14 ± 0.80^{ab}	72.47 ± 0.35^{a}	70.49 ± 0.36^{bc}	**	69.59 ± 0.19	72.74 ± 0.26	**
Cold car.	69.94 ± 0.65^{a}	66.23 ± 0.53^{d}	68.25 ± 0.65^{abc}	66.91 ± 0.64^{bcd}	68.43 ± 0.48^{ab}	66.42 ± 0.72^{cd}	**	65.69 ± 0.23	69.71 ± 0.23	**

NSD-CONT: normal stocking density and no additive HSD- KONT: high stocking density (HSD) and no additive, HSD-ANT: HSD and 10 mg/kg of avilamycin additive, HSD-T1, HSD-T2, and HSD-T3: HSD and 200, 400, 600 mg/kg of volatile oil additive, respectively.
* Differences between the averages indicated by different letters in the same line are statistically significant ($P < 0.01$).
** Differences between the averages indicated by different letters in the same row are statistically significant ($P < 0.05$).

160.89 ± 3.63 g and 153.57 ± 3.81 g respectively, which were significantly lower than the other groups ($P < 0.01$). It was determined that there was no significant difference between female and male hot and cold carcass weights ($P > 0.05$). The lowest hot carcass yield was in HSD - CONT group (69.41 ± 0.30%), and the highest hot carcass yield was found in HSD - T2 group (72.47 ± 0.35%). Similarly, the lowest cold carcass yield was 66.23 ± 0.53% in HSD - ANT group. The effect of sex on hot and cold carcass yield was found to be significant ($P < 0.01$).

Table 4. Effect of thyme volatile oil addition to quail rations on intestinal microflora (cfu/g).

	NSD-CONT	HSD- KONT	HSD-ANT	HSD-T1	HSD-T2	HSD-T3	P
Total bacteria	8.45 ± 0.11^{ab}	8.78 ± 0.16^{a}	7.49 ± 0.25^{cd}	8.61 ± 0.12^{a}	7.89 ± 0.29^{bc}	6.97 ± 0.33^{d}	**
Coliform	5.00 ± 0.22^{a}	4.40 ± 0.12^{ab}	3.48 ± 1.07^{c}	4.46 ± 0.15^{ab}	4.14 ± 0.22^{b}	2.54 ± 0.29^{d}	**
Lactobacillus	3.87 ± 0.17^{a}	3.77 ± 0.17^{a}	3.07 ± 0.17^{bc}	3.54 ± 0.18^{ab}	3.47 ± 0.20^{ab}	2.84 ± 0.21^{c}	**

NSD-CONT: normal stocking density and no additive HSD- KONT: high stocking density (HSD) and no additive, HSD-ANT: HSD and 10 mg/kg of avilamycin additive, HSD-T1, HSD-T2, and HSD-T3: HSD and 200, 400, 600 mg/kg of volatile oil additive, respectively.
** Differences between the averages indicated by different letters in the same line are statistically significant (P < 0.01).

The total bacterial, coliform, and lactobacillus levels (cfu/g) in the small intestine microbiota were significantly affected by the stocking density and the additives within the feed (P < 0.01). Lactobacillus levels were similar in NSD - CONT (3.87 ± 0.17), HSD - CONT (3.77 ± 0.17), HSD - T1 (3.54 ± 0.18), and HSD - T2 (3.47 ± 0.20); and they were particularly higher than they were in HSD - T3 (2.84 ± 0.21) (P < 0.01). The highest lactobacillus level (3.87 ± 0.17) was found in the positive control (NSD - CONT) group, while the lowest value (2.84 ± 0.21) was observed in the group to whose diet 600 mg/kg of volatile oil was added (HSD - T3). A significant decrease was observed in the total bacterial counts of groups HSD - T2 (7.89 ± 0.29) and HSD - T3 (6.97 ± 0.33), to whose diets thyme extract was added (P < 0.01). Similarly, the coliform number of HSD - T3 group (2.54 ± 0.29), with 600 mg/kg of volatile oil supplementation, was significantly lower than that of the other groups. It was determined that increased levels of thyme extract (HSD - T2, 4.14 ± 0.22) significantly suppressed coliform number (P < 0.01).

4. Discussion

Based on the in vitro studies conducted on volatile oils and their components as well as the active ingredients in their structure, they have the potential to act as an alternative to antibiotics due to their antimicrobial effect, which is claimed to have positive effects on many systems in the living. The mixtures formed have a synergistic effect, and the most significant synergistic effect appeared in the combination of thymol and carvacrol [11,12]. Also, it has been stated that the synergistic effects of active ingredients, such as ocimene and terpinene are also important, especially carvacrol among the active compounds [13]. At the end of this study, it was observed that the live weight gain of all groups was improved compared to the negative control group (HSD - CONT), and this improvement was found to be statistically significant (P < 0.01). This result showed that the stocking density had a negative effect on animal performance and that adding avilamycin and thyme volatile oil to feeds reduced the negative effect of stocking density. These findings are in parallel to those of other studies reporting that volatile oils have a positive effect on weight gain [14,15]. In order to obtain the best results in terms of feed consumption and live weight gain regarding the feeding ration of animals, it is extremely important to know the exact composition and formulation of the feed additive to be used [16]. In a study on the effect of zahter volatile oil on Japanese quails, Aksu et al. [17] reported that 600 mg/kg of volatile oil showed the highest live weight gain. In contrast to the studies reporting that the stocking density decreased the live weight [3,18,19,20], Kaynak et al. [21] reported that the stocking density had no significant effect on the live weight gain. Yörük et al. [22] stated that a decreasing henhouse area, which means increasing stocking density, had a negative effect on egg yield and weight, live weight, and feed conversion ratio (P < 0.01).

The effect of density on feed conversion ratio was found to be significant in the study (P < 0.01). Because the negative control group (HSD - CONT) animals consumed significantly (P < 0.01) less feed than the other groups, their live weights were significantly lower compared to the other groups, which had a negative effect on the feed conversion ratio (P < 0.01). Data on feed consumption during the study show that as the stocking density increased, feed consumption decreased, and the difference between the groups was statistically significant (P < 0.01). This situation is compatible with the work on broilers by Alçiçek et al. [23], Ravindran et al. [24], and Skomorucha et al. [25]. However, with a decrease in stocking density, feed consumption increased but without statistical significance [26]. Also, there are literature reports indicating that a high stocking density decreased the air circulation for the animals at the back of the poultry house and reduced the heat transfer from the surface of the litter, thus decreasing the heat loss from the body of the animals [27,28].

Aksu et al. [17] observed that feed conversion ratio was not affected by the addition of thyme oil at 200, 400, and 600 mg/kg level in quails fed in normal stocking density. Lee et al. [29] added 200 mg/kg of carvacrol to broiler ration, but they found a decrease in feed consumption. They observed that the addition of 200 mg/kg of carvacrol to broiler

ration increased feed conversion ratio despite a decrease in feed consumption. They concluded that it may be due to an increase in energy and nutrient conversion. Some studies investigated the effects of stocking density on feed conversion ratio, and the effect of the frequency of feeding on the feed conversion ratio was reported to be insignificant [3,21,30–32]. However, in some studies [33,34], it was reported that the use of feed is adversely affected. The reason for the decrease in the performance values of the animals in high stocking density is that as the animals grow, there is increased ambient temperature and difficulty in reaching water due to the reduction in the unit area, which causes stress [35,36]. The differences in the performance values obtained in various research studies are due to the plant species used to produce the volatile oils, physical and chemical soil conditions, harvest time, plant ripening degree, drying method, storage time, and extraction process, which is influenced by factors such as the chemical components of the volatile oil and the mechanism of action [37,38].

At the end of our experiment, the difference between the hot and cold carcass weights was statistically significant ($P < 0.01$), and the effect of sex was insignificant. The hot and cold carcass weights of the negative control group (HSD - CONT) were significantly lower than those of the other groups ($P < 0.01$). This result is consistent with the study by Alçiçek et al. [39], which found that adding volatile oil containing plant extracts to feed affected the carcass weight and conversion positively. However, in a study involving 10 broilers/m², 13 broilers/m², and 16 broilers/m² stocking density, Kaynak et al. [21] observed that the effect of stocking density on hot and cold carcass weights was not significant, while the effect of sex on hot and cold carcass weights was significant ($P < 0.05$).

When the intestinal microbiota load was examined (Table 4), it was seen that coliform, lactobacillus, and total bacteria levels were statistically significantly affected in all the groups ($P < 0.01$), and the thyme volatile oil showed linear antibacterial effects. Similarly, Aksu et al. [17] reported that adding zahter-tyhme volatile oil to quail rations induced a statistically significant difference ($P < 0.01$) in intestinal microbiota *Escherichia coli* total bacterial level. Güler et al. [40] examined the effects of thyme, anise oil, and conventional antibiotics on the total number of colonic bacteria in the intestine of broilers. They concluded that there was a decrease in the number of fecal coliforms compared to the untreated control group in all experimental groups, and this difference was statistically significant except for thyme - 200 ppm group ($P < 0.01$). These findings show complete parallelism with our study regarding the addition of 600 mg/kg of volatile oil supplement (HSD - T3).

In conclusion, it was determined that high stocking density (90 cm/quail) increased the stress level of the animals, which adversely affected their performance, and positive results were obtained in all the groups in which different doses of zahter-tyhme volatile oil were applied. Total bacterial density, coliform, and lactobacillus counts in intestinal microbiota were significantly affected by the stocking density and feed additives. As a result, volatile thyme oil supplementation in the ration of high stocking density quails improved the intestinal microbiota and performance parameters at different levels. It was concluded that, particularly, 600 mg/kg of zahter-thyme volatile oil supplementation was more effective in lessening the adverse effects caused by high stocking density.

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