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Evaluating the Effect of Two Types of Thyme Essential Oils (*Zataria Multiflora* & *Ziziphora Clinopodioides* Lam) on Some Productive Traits and Blood Parameters in Broilers

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Abstract

This experiment was conducted to study the effect of thyme extracts from *Zataria multiflora* and *Ziziphora clinopodioides* Lam on some productive traits and blood parameters. A total of 240 one-day-old, male broilers of Ross 308 were used in a completely randomized design with 6 experimental groups and 4 replicates with 10 birds in each. Experimental treatments consisted of a basal soybean-meal diet (control), the basal diet supplemented with vitamin E at 200 mg/kg, *Zataria multiflora* essential oil at 200 mg/kg or 400 mg/kg, and *Ziziphora clinopodioides* essential oil at 200 mg/kg or 400 mg/kg. The results showed that the birds in the experimental groups consumed more feed and had significantly greater body weight as well as energy and protein efficiency in the whole experimental period, especially *Ziziphora clinopodioides* at the level of 400 mg/kg, compared to the control group ($P < 0.05$). Thyme extracts had a significant effect on the most blood factors, except cholesterol and LDL-c ($P < 0.05$). Thyme extracts significantly reduced serum albumin and improved HDL-c level ($P < 0.05$). The experimental groups had a significant effect on the relative weight of breast and thigh, malondialdehyde concentration, lipid peroxidation, and antioxidant enzyme activity ($P < 0.05$). Based on the results, *Ziziphora clinopodioides* at the level of 400 mg/kg can be introduced as an effective oral additive to improve the studied traits.

Introduction

Dietary supplement of antibiotics exhibits beneficial effects on chicken health and performance, but their application has been prohibited due to its harmful effects on the consumers and causing antibiotic-resistant in bacteria (Miles *et al*., 2006; Mehdi *et al*., 2018; Hamid *et al*., 2019; Roth *et al*., 2019; Van *et al*., 2020). By eliminating growth-promoting antibiotics from poultry diets, medicinal plants and their various derivatives were the subjects of research by many researchers to introduce safer alternatives to antibiotics (Bedford, 2000). Most herbal supplements can improve the performance and functions of the immune system due to their antioxidant properties. These compounds may improve the bird's resistance to disease by directly stimulating the immune system. They can also alter cholesterol metabolism, producing a healthier product for human consumption (Durape, 2007).

One of the important antioxidants is vitamin E. Vitamin E is a fat-soluble vitamin with plant origin, which is essential for the proper functioning of the reproductive (Mohd Mutalip *et al*., 2018), nervous (Sen *et al*., 2004), muscular (Rizvi *et al*., 2014), and immune systems (Lee and Han, 2018). This vitamin strengthens the immune system by affecting the immune cells (Lewis *et al*., 2019), endocrine (Huang *et al*., 2019), and metabolic systems (Schmölz *et al*., 2016), macrophage alienation (Sakamoto *et al*., 1999), and antibody production (Lee and Han, 2018). Vitamin E increases the body's immune function by reducing the production of prostaglandin PGE 2 as a factor of the immune system (Lee and Han, 2018).

Researchers have now begun to use natural alternatives that are both effective in eliminating harmful effects on birds and humans and more affordable. Herbal supplements are one of them which should not only be considered as alternatives to growth-promoting antibiotics but also have beneficial properties that growth-promoting antibiotics lack. Plant essential oils and organic acids with growth-promoting and also antimicrobial effects are effective

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alternatives to the antibiotics ((Isabel and Santos, 2009; Yang *et al*., 2018 and 2019; Xu *et al*., 2018; Giannenas *et al*., 2019; Oso *et al*., 2019; Dev *et al*., 2020; Galli *et al*., 2020). For instance, Thyme essential oil is an additive with beneficial effects on weight gain and body mass index (Abdel-Wareth *et al*., 2012; Dehghani *et al*., 2018; Kheiri *et al*., 2018; El-Ashram and Abdelhafez, 2020). Although thyme oil extract has been proven as a booster of the immune system (Ragaa *et al*., 2016), there is no information about its effectiveness on the growth performance of broiler chickens.

Medicinal plants contain two classes of active ingredients. The first class is carbohydrates that are produced in all green plants by photosynthesis and used for primary metabolism. The second class consists of essential oils, resins, and various alkaloids that are produced by plant nitrogen uptake and involved in secondary metabolism (Wang *et al*., 2017; Hao and Xiao, 2020). The latter group is often helping plants survive and their therapeutic effects are well documented in human health (Paultre *et al*., 2021). Generally, these compounds are not found in pure form but are combined with other components that complement their effects (Dhifi *et al*., 2016).

Thyme contains 6.2 to 8 percent of essential oils, most of which are phenols, monoterpene hydrocarbons, and alcohols (Bozkurt *et al*., 2016). Thymol is a major phenolic compound but the most important effect of thyme is due to the presence of its main active ingredient which is called carvacrol (Felici *et al*., 2020). Carvacrol has antioxidant effects and is useful in controlling poultry diseases (Felici *et al*., 2020). Due to the need to pay attention to the role of medicinal plants in promoting the immune system and performance of poultry, this experiment was performed to evaluate the effect of essential oils of *Zataria multiflora* and *Ziziphora clinopodioides* on yield, carcass characteristics, some blood parameters, and antioxidant indices of broilers.

Materials and Methods

Birds and Experimental Design

In this study, 240 one-day-old male broilers of Ross 308 were randomly divided into 6 groups with 4 replicates and 10 chicks per replication in a completely randomized design (CRD). During the experiment, the light regime was 23 hours and one hour of darkness.

Table 1. The constituents of the essential oils (%) of *Zataria multiflora* and *Ziziphora clinopodioides*

Compound	*Zataria multiflora*	Compound	*Ziziphora clinopodioides*
α-Thujene	0.32	α-Thujene	0.13
α-Pinene	2.24	α-Pinene	1.20
Camphene	0.12	Camphene	0.80
Sabinene	0.34	Sabinene	2.21
β-Pinene	0.05	β-Pinene	0.06
1-Octen-3-ol	0.64	Myrcene	0.32
Myrcene	1.03	p-Cymene	0.06
3-Octanone	0.13	α-Terpipene	0.05
α-Phellandrene	0.15	1,8-Cineole	7.89
α-Terpipene	0.83	γ-Terpinene	0.22
p -Cymene	7.15	Cis-Sabinene hydrate	0.46
1,8-Cineole	0.23	Terpinolene	0.29
γ-Terpinene	3.63	Linalool	0.64
Cis-Sabinene hydrate	0.12	Camphore	0.05
Terpinolene	0.13	P-menth-3-en-8-ol	10.42
Linalool	0.89	Menthone	19.47
Terpinene-4-ol	0.22	Menthofuran	1.22
α-Terpine	0.32	Neomenthol	1.13
Trans-dihydrocarvon	0.99	Menthol	7.24
Thymyl methyl ether	2.18	Isomenthol	0.77
Carvacrol methyl ether	0.26	Pulegone	23.06
Thymol	32.92	Piperitone	6.25
Carvacrol	39.94	Piperitenene	2.90
Thymyl acetate	0.39	Neomenthyl acetate	4.24
Carvacryl acetate	0.58	Bornylacetate	0.82
β - Caryophyllene	2.37	β-Bourbonene	3.75
Aromadendrene	0.34	β - Caryophyllene	0.23
α-Humulene	0.10	Germcren-D	0.39
Bicyclogermacrene	0.05	α-Humulene	0.21
Germcren-D	0.24	Bicyclogermacrene	0.11
Spathulenol	0.23	(E)-α-Bisabolen	0.06
Caryophyllene oxide	0.39	Spathulenol	0.40
		Caryophyllene oxide	2.48
Total (%)	99.54	Total (%)	99.53

Extraction and measurement of essential oils

The two types of thyme, *Zataria multiflora*, and *Ziziphora clinopodioides Lam* were purchased from the local market and identified through Shiraz University experts. Then collected aerial parts were dried under laboratory conditions (25°C for 15 days). After drying, the plants were carefully ground and essential oil was extracted via g Hydro-distillation Clevenger. The essential oils were collected for 3 hours from the time of boiling and the resulting essential oils were wrapped in a sealed container. The gas chromatography device was used to separate the compounds using the FID detector. After preparation, the essential oils of the plants were injected into the GC/MS device to determine the type of their constituent compounds (Table 1).

Experimental diets

A corn-soybean meal based diet was formulated according to Ross 308 requirements for starter, grower, and finisher periods by UFFDA software (Table 2). The experimental diets were prepared by supplementing appropriate amounts of vitamin E or essential oils to the basal diets; including: 1- Basal diet (BD), 2- Basal diet + Vitamin E (200 mg/kg; VitE200), 3- Basal diet + *Zataria multiflora* (200 mg/kg; ZM200), 4- Basal diet + *Zataria multiflora* (400 mg/kg; ZM400), 5- Basal diet + *Ziziphora clinopodioides* (200 mg/kg; ZC200), 6- Basal diet + *Ziziphora clinopodioides* (400 mg/kg; ZC400). The feed was monitored several times during the day and provided to the chickens properly with appropriate drinking water (*ad-libitum*).

Table 2. Ingredients and nutrients composition of the basal diet

Item (%)	1-10 days (Starter)	11-24 days (Grower)	25-42 days (Finisher)
Ingredients			
Corn	49.67	53.08	58.37
Soybean meal (440 g CP/kg)	41.66	37.87	32.39
Soybean oil	4.40	5.28	5.82
DL-methionine	0.39	0.33	0.30
L-lysine-HCl	0.21	0.14	0.14
L-threonine	0.09	0.05	0.03
Dicalcium phosphate	2.20	1.95	1.71
Calcium carbonate	1.04	0.96	0.90
Common salt	0.29	0.29	0.29
Vitamin-mineral premix[a]	0.05	0.05	0.05
Calculated composition			
Metabolizable energy (kcal/kg)	3000	3100	3200
Crude protein	23.00	21.50	19.50
Methionine +Cystine	1.08	1.00	0.90
Lysine	1.44	1.30	1.15
Threonine	0.97	0.88	0.79
Calcium	0.96	0.87	0.79
Available phosphorus	0.48	0.44	0.39
Analyzed composition[b]			
Gross energy (kcal/kg)	4040	4090	4120
Dry matter	91.59	91.50	91.30
Crude protein	22.57	21.02	18.98
Ether extract	6.86	7.80	8.44
Neutral detergent fiber	10.68	10.55	10.40
Acid detergent fiber	4.53	4.32	4.03
Ash	6.94	6.44	5.86
Calcium	1.09	1.00	0.89
Total phosphorus	0.76	0.70	0.64

[a]The vitamin-mineral premix provided the following quantities per kg of diet: vitamin A, 10,000 IU (all-trans-retinal); cholecalciferol, 2,000 IU; vitamin K3, 3.0 mg, thiamin, 1.1 mg; riboflavin, 18.0 mg; niacin, 50 mg; D-calcium pantothenic acid, 24 mg; vitamin B6, 2.94 mg; biotin, 0.5 mg; choline chloride, 450 mg; vitamin B12, 0.02 mg; folic acid, 3.0 mg; manganese (as MnSO4•H2O), 110 mg; iron (as FeSO4•7H2O), 60 mg; zinc (as ZnO), 90 mg; copper (as CuSO4), 10 mg; iodine (as Ca(IO3)2), 0.46 mg; selenium (as Na2SeO3), 0.2 mg.

[b]Dry matter (method 934.01), crude protein (method 954.01), ether extract (method 920.39), ash (method 942.05), calcium (method 968.08), and phosphorus (method 965.17) were determined as per AOAC (2000) and gross energy was measured by an Adiabatic Bomb Calorimeter (Gallenkamp autobomb, Leicestershire, UK). Neutral detergent fiber and acid detergent fiber were determined according to the procedures of Van Soest *et al*. (1991), and sodium sulfite was used in the assay.

Measurement of parameters

Feed efficiency

At the end of 24 and 42 days, the chicks of each pen were weighed, and also the feed consumption was measured. By calculating the difference in weight of chickens at the end and beginning of each period, the amount of weight gain in that period was calculated, and the feed conversion ratio was determined. Before weighing, the birds were starved for 4 hours to empty the contents of the gastrointestinal tract. During the experimental period, losses or eliminated chickens were recorded daily. Energy and protein efficiencies were also calculated for the grower, finisher, and whole period (Nasr *et al*., 2011) as below:

Energy efficiency= (Weight gain $_g$/Metabolic energy consumption $_{kcal}$) × 100

Protein efficiency= (Weight gain $_g$/Protein consumption $_g$) × 100

Carcass parameters

In this experiment, carcass traits were measured at the end of the three stages: starter (10 days), grower (24 days), and finisher (42 days). To evaluate the carcass characteristics, one bird was slaughtered from each pen whose average weight was close to the average weight of the same pen. Consumable carcass weight along with internal organs' weight including breast, thighs, wings, gizzard, abdominal fat, lungs, viscera, small intestine, heart, liver, spleen, and bursa Fabricius were measured. Simultaneously with determining the carcass efficiency, abdominal fat, fat around the heart, gizzard, liver, and intestines were also determined separately.

Blood biochemistry

3 mL of blood was taken from the brachial vein of each chicken and poured into labeled test tubes and then transferred to the laboratory for serum preparation. The samples were centrifuged at 700 g for 15 minutes. The protocol and commercial kits of Pars Azmun Company (S2100 Series UV/Vis, Spectrophotometer) were used for determining the concentrations of glucose, triglyceride, albumin, globulin, cholesterol, HDL-c, LDL-c, total phosphorus, uric acid, and hemoglobin. Hematocrit percentage was also determined using a hematocrit ruler. The serum was poured into a microtube and stored in a freezer at -20°C before transferring to the laboratory.

Antioxidant assay

To evaluate the antioxidant status and the lipid peroxidation, at the end of all periods, two chickens were selected from each pen and blood samples were taken from their brachial vein. The samples were transferred to the laboratory in heparinized tubes and immediately centrifuged for isolation of red blood cells. TBARS test was used to measure the amount of malondialdehyde as an indicator of oxidation in blood plasma. First, 50 mL of reagent was prepared using 7.5 mM of trichloroacetic acid + 187 mM of TBA + 6.25 mL of hydrochloric acid (2 nanomoles per liter) via mixing and placing in a boiling water jar to dissolve well. Then, 3 mL of the prepared reagent with 300 µL of each plasma sample were poured into the test tubes with a lid and placed in a snake bin. The amount of 2 mL of isobutanol was added to each test tube and mixed with a mixer for 20 seconds. After centrifugation, the samples were centrifuged for 10 minutes at 700 g (refrigerated centrifuges) and read at 532 wavelengths (Placer *et al.*, 1996).

Serum SGPT and SGOT values were measured by an autoanalyzer (BioSystems S. A. Costa Brava 30.08030 Barcelona, Spain). The activity of glutathione peroxidase and superoxide dismutase was also measured using the whole blood containing EDTA with diluted Drabkin reagent. For this purpose, according to the kit protocol, hemolyzes were prepared, and then the preparation was performed using kit regents. The decrease in absorbance at 340 and 5050 nm was measured by a spectrophotometer.

In this experiment, the RANSOD-RANSElL commercial kit was used. The amount of fat peroxidation in breast tissue was measured by determining the amount of TBARS. For this assay, a total of 40 µL of homogenized tissue was added to 40 µL of 0.9% sodium chloride and 40 µL of distilled water and placed at 37° C for 20 minutes. The reaction was then stopped using 600 µL of hydrochloric acid 0.8 mol containing 12.5% trichloroacetic acid. After adding 780 µL of 1% thiobarbituric acid, the solution was boiled for 20 minutes and cooled to 4° C. The cold solution was centrifuged at 252 g for 20 minutes and then its light absorption at 532 nm vs. Blank solution was used to calculate the amount of TBARS using a reactive quenching factor of 1.5×10^5/Cm×mol. Thiobarbituric acid was calculated as an indicator of lipid peroxidation (expressed as nmol/g tissue protein; Alirezaei *et al*., 2012).

Statistical Analyses

Data were processed in Excel and analyzed using the ANOVA procedure of SAS 9.1. The mean values were compared by Duncan's multiple comparison tests. The statistical model was:

$Y_{ij} = \mu + T_i + e_{ij}$

Y_{ij} = value of each observation, μ = average, T_i = effect of i treatment and e_{ij} = error

Results

The results in Table 3 show that there was a significant difference between feed intakes in different periods.

Table 3. The effect of thyme extracts and vitamin E on feed intake, weight gain, feed conversion ratio, energy and protein efficiency ratio of broilers

Treatments	Feed intake (g)			Weight Gain (g)			FCR[8]			Energy efficiency (%)			Protein efficiency (%)		
	0-24	25-42	0-42	0-24	25-42	0-42	0-24	25-42	0-42	0-24	25-42	0-42	0-24	25-42	0-42
Control[1]	1706.01^{b}	2992.05^{b}	4698.06^{c}	1220.03^{b}	1144.00^{c}	2364.03^{c}	1.39	2.61^{a}	1.99^{a}	23.43	12.00^{b}	16.49^{c}	3.19	1.93^{b}	2.25^{b}
VitE200[2]	1727.44^{ab}	3017.69^{ab}	4745.13^{b}	1224.58^{b}	1174.50^{bc}	2399.08^{c}	1.41	2.57^{ab}	1.98^{a}	23.22	12.22^{b}	16.56^{b}	3.16	1.97^{b}	2.26^{b}
ZM200[3]	1750.41^{ab}	3069.23^{a}	4819.64^{ab}	1303.25^{a}	1246.50^{bc}	2549.75^{b}	1.34	2.46^{ab}	1.89^{ab}	24.39	12.75^{b}	17.33^{b}	3.32	2.05^{ab}	2.36^{a}
ZM400[4]	1812.60^{a}	3123.03^{a}	4935.63^{a}	1303.58^{a}	1275.75^{b}	2579.33^{b}	1.39	2.45^{ab}	1.91^{ab}	23.56	12.82^{b}	17.12^{b}	3.21	2.07^{ab}	2.33^{a}
ZC200[5]	1753.05^{ab}	3085.09^{a}	4838.14^{ab}	1228.63^{b}	1242.75^{b}	2471.38^{bc}	1.43	2.48^{ab}	1.96^{a}	22.96	12.64^{b}	16.73^{b}	3.13	2.04^{ab}	2.28^{ab}
ZC400[6]	1800.11^{a}	3097.29^{a}	4897.40^{ab}	1267.40^{ab}	1442.00^{a}	2709.40^{a}	1.42	2.14^{b}	1.81^{b}	23.07	14.61^{a}	18.12^{a}	3.14	2.35^{a}	2.47^{a}
SEM[7]	29.11	53.00	36.58	25.55	56.13	52.03	0.03	0.11	0.03	0.21	0.41	0.56	0.03	0.39	0.08
P-value	0.0199	0.030	0.034	0.039	<0.000	<0.000	0.0176	<0.000	0.042	0.097	0.0139	0.048	0.096	0.0138	0.038

[1] Basal diet, [2] Basal diet + Vitamin E (200mg/kg), [3] Basal diet + *Zataria multiflora* (200 mg/kg), [4] Basal diet + *Zataria multiflora* (400 mg/kg), [5] Basal diet + *Ziziphora clinopodioides* (200 mg/kg), [6] Basal diet + *Ziziphora clinopodioides* (400 mg/kg), [7] Standard error of the mean [8] Feed conversion ratio.

Numbers with dissimilar letters in each column have a statistically significant difference ($P < 0.05$).

The *Ziziphora clinopodioides* extract (400 mg/kg) increased feed intake to 1812.60g (0-24 days), followed by 3123.03g (25-42 days), and 4935.63g in the whole period. The outcomes revealed that there were significant differences between weight gain in 0-24, 25-42 days, and the whole period of the experiment ($P < 0.05$). Considering the whole period, the highest weight gain (2709.40 g) was only obtained in ZC400 group. While the calculated feed conversion ratio also showed a significant difference at the age of 21-42 days and the whole period. Using the higher level of *Ziziphora clinopodioides* extract (400 mg/kg), the feed conversion ratio improved by 0.18 units.

The results showed that thyme extracts had a significant effect on energy and protein efficiency at the age of 25-42 days and the whole period. Using thyme extracts, energy efficiency increased from 12% to 14.61% in 25-42 days, and in the whole period, the highest amount of energy efficiency belonged to the 400 mg/kg *Zataria multiflora* essential oil group (18.12%). Consumption of thyme extracts at the age of 25 to 42 days increased the protein efficiency so that consumption of *Zataria multiflora* essential oil (400 mg/kg) (2.35%) recorded the highest energy efficiency at this age (Table 3).

Thyme extracts showed a significant influence on most blood parameters except cholesterol and LDL-c. Adding the extracts reduced the amount of albumin, and improved HDL-c concentration ($P < 0.01$) (Table 4).

Table 4. Effect of thyme extracts and vitamin E on blood biochemical parameters of broilers

Treatments	Albumin (g/dL)	Cholesterol (mg/dL)	HDL-c (mg/dL)	Glucose (mg/dL)	TP[8] (mg/dL)	Uric acid (mg/dL)	TG[9] (mg/dL)	LDL-c (mg/dL)	Globulin (g/dL)	Hematocrit (%)	Hb[10] (g/dL)
Control[1]	4.18^{a}	173.00	25.53^{c}	192.30^{a}	6.07^{a}	7.28^{ab}	150.64^{b}	80.76	1.94^{a}	44.75^{a}	16.08^{b}
VitE200[2]	3.09^{b}	173.03	45.67^{b}	178.26^{b}	5.72^{ab}	7.33^{ab}	139.16^{c}	94.17	1.67^{b}	35.50^{b}	14.25^{bc}
ZN200[3]	3.12^{b}	175.73	52.33^{a}	185.68^{ab}	5.87^{ab}	7.57^{ab}	148.79^{b}	98.96	1.56^{c}	39.25^{ab}	13.77^{bc}
ZM400[4]	3.35^{b}	183.31	49.51^{ab}	193.29^{a}	6.06^{a}	8.09^{a}	160.69^{a}	95.48	1.97^{a}	44.00^{a}	21.02^{a}
ZC200[5]	3.17^{b}	180.19	49.44^{ab}	184.^{27ab}	5.56^{b}	6.92^{b}	152.91^{ab}	78.67	1.47^{d}	36.50^{b}	14.26^{bc}
ZC400[6]	3.15^{b}	179.64	49.95^{ab}	160.51^{c}	5.88^{ab}	7.25^{ab}	155.46^{ab}	98.74	1.77^{b}	40.00^{ab}	27.24^{a}
SEM[7]	0.06	6.15	1.74	4.25	0.15	0.29	5.28	9.01	0.17	2.27	1.67
P-value	0.006	0.694	0.002	0.002	0.001	0.007	0.0001	0.643	0.0001	0.0005	0.001

[1] Basal diet, [2] Basal diet + Vitamin E (200 mg/kg), [3] Basal diet + *Zataria multiflora* (200 mg/kg), [4] Basal diet + *Zataria multiflora* (400 mg/kg), [5] Basal diet + *Ziziphora clinopodioides* (200 mg/kg), [6] Basal diet + *Ziziphora clinopodioides* (400 mg/kg), [7] Standard error of the mean, [8] Total phosphorus, [9] Triglyceride, [10] Hemoglobin.

Numbers with dissimilar letters in each column have a statistically significant difference ($P < 0.05$).

Table 5. Effect of different concentrations of thyme extracts and vitamin E on the relative carcass weight and internal organs of broilers in the starter period (g/kg live body weight)

Relative weight	Treatments							
	Control[1]	VitE200[2]	ZN200[3]	ZM400[4]	ZC200[5]	ZC400[6]	*SEM*[7]	*P*-value
Body weight (g)	795.11	821.16	834.66	839.83	830.76	835.66	57.41	0.112
Consumable carcass	621.13	624.25	627.22	627.96	626.62	627.38	18.17	0.624
Breast	329.76	331.16	332.10	337.33	331.43	338.66	48.36	0.116
Thigh	213.48	233.41	243.58	257.25	241.48	254.04	35.51	0.362
lung	16.37	17.01	17.47	18.29	17.09	17.71	2.39	0.87
Liver	16.41	16.88	16.35	17.24	16.33	16.67	0.45	0.756
Gizzard	39.93	39.73	35.33	42.36	28.86	38.06	1.25	0.157
Intestine	70.03	69.58	69.22	69.96	70.28	70.38	1.74	0.378
Intestinal fat	9.96	7.00	7.93	8.70	8.39	8.02	1.74	0.378
Fat around the heart	1.38	1.34	1.86	1.20	0.70	0.84	0.64	0.96
Liver fat	3.04	3.02	2.48	3.07	2.81	3.04	0.75	0.768
heart	18.66	19.08	20.16	20.83	19.81	20.40	0.69	0.596
viscera	56.13	55.89	53.41	54.78	54.41	53.45	7.21	0.547
Abdominal fat	28.33	28.08	26.78	27.58	26.82	27.74	3.47	0.432
Wings	0.92	0.91	0.93	0.85	0.93	0.85	0.62	0.658
Spleen	2.60	2.72	3.09	3.22	3.09	3.04	0.78	0.106
Bursa Fabricius	0.69	0.74	0.62	0.84	0.89	0.81	0.75	0.37
Gizzard fat	9.35	6.97	6.92	7.61	7.19	6.80	2.10	0.298

[1] Basal diet, [2] Basal diet + Vitamin E (200 mg/kg), [3] Basal diet + *Zataria multiflora* (200 mg/kg), [4] Basal diet + *Zataria multiflora* (400 mg/kg), [5] Basal diet + *Ziziphora clinopodioides* (200 mg/kg), [6] Basal diet + *Ziziphora clinopodioides* (400 mg/kg), [7] Standard error of the mean.

Table 6. Effect of different concentrations of thyme extracts on the relative carcass weight and internal organs of broilers in the grower period (g/kg live body weight)

Relative weight	Treatments							
	Control[1]	VitE200[2]	ZN200[3]	ZM400[4]	ZC200[5]	ZC400[6]	*SEM*[7]	*P*-value
Body weight (g)	1590.22^{c}	2502.33^{a}	2483.33^{a}	2289.66^{b}	2431.33^{a}	2503.53^{a}	68.25	0.017
Consumable carcass	1242.27	1248.50	1254.45	1255.93	1253.24	1254.77	20.34	0.514
Breast	659.53^{b}	662.33^{b}	664.20^{b}	674.66^{b}	662.86^{b}	877.33^{a}	6.34	0.015
Thigh	426.97^{d}	466.83^{c}	487.16^{bc}	514.50^{b}	482.96^{bc}	608.28^{a}	1.67	0.001
lung	32.75^{b}	34.02^{ab}	34.94^{ab}	36.59^{ab}	34.18^{ab}	42.35^{a}	3.10	.032
Liver	32.83	33.76	32.71	34.48	32.66	33.29	0.17	0.635
Gizzard	79.86	79.46	70.66	84.73	57.73	76.13	10.09	0.387
Intestine	140.07	139.17	138.45	139.93	140.57	140.77	12.33	0.468
Intestinal fat	19.92	14.00	15.86	17.40	16.78	16.05	3.21	0.374
Fat around the heart	2.77	2.68	2.73	2.41	1.40	1.68	0.32	0.825
Liver fat	6.08	6.04	4.96	6.14	5.63	6.08	0.51	0.965
heart	37.33	38.16	40.33	41.66	39.62	40.80	0.87	0.478
viscera	100.00	114.16	114.66	114.26	115.05	113.61	17.21	0.287
Abdominal fat	1.84	1.82	1.86	1.70	1.86	1.70	0.15	0.845
Wings	1.84	1.82	1.86	1.70	1.86	1.70	0.35	0.845
Spleen	3.97^{c}	5.44^{b}	6.18^{ab}	6.44^{a}	6.18^{ab}	6.09^{ab}	0.10	0.032
Bursa Fabricius	1.38	1.48	1.24	1.68	1.79	1.63	0.24	0.485
Gizzard fat	18.70	13.94	13.85	15.22	14.38	13.61	2.12	0.365

[1] Basal diet, [2] Basal diet + Vitamin E (200mg/kg), [3] Basal diet + *Zataria multiflora* (200 mg/kg), [4] Basal diet + *Zataria multiflora* (400 mg/kg), [5] Basal diet + *Ziziphora clinopodioides* (200 mg/kg), [6] Basal diet + *Ziziphora clinopodioides* (400 mg/kg), [7] Standard error of the mean.

Numbers with dissimilar letters in each row have a statistically significant difference ($P < 0.05$).

Table 7. Effect of different concentrations of thyme extracts on the relative carcass weight and internal organs of broilers in the finisher period (g/kg live body weight)

Relative weight	Treatments							
	Control[1]	VitE200[2]	ZN200[3]	ZM400[4]	ZC200[5]	ZC400[6]	*SEM*[7]	*P*-value
Body weight (g)	2364.03^{c}	2399.0^{8c}	2549.75^{b}	2579.33^{b}	2471.38^{bc}	2709.40^{a}	56.13	0.000
Consumable carcass	1863.41	1872.76	1881.68	1883.90	1879.86	1882.16	22.33	0.458
Breast	989.30^{b}	993.50^{ab}	996.30^{ab}	1012.00^{a}	994.30^{ab}	1016.10^{a}	10.76	0.029
Thigh	640.46^{b}	700.25^{ab}	730.75^{a}	762.12^{a}	724.45^{a}	771.75^{a}	1.67	0.009
lung	49.13^{b}	51.04^{ab}	52.42^{ab}	54.89^{ab}	51.27^{ab}	63.14^{a}	2.92	0.048
Liver	49.13^{b}	51.04^{b}	52.42^{ab}	54.89^{ab}	51.27^{b}	63.14^{a}	2.92	0.048
Gizzard	119.80	119.2	106.00	127.10	86.60	114.20	11.06	0.245
Intestine	210.11	208.76	207.68	209.90	210.86	211.16	22.33	0.458
Intestinal fat	29.88	21.00	23.79	26.11	25.18	24.08	2.13	0.211
Fat around the heart	4.16	4.02	5.60	3.62	2.11	1.53	0.76	0.726
Liver fat	9.12	9.06	7.44	9.22	8.45	9.12	0.31	0.898
heart	56.00	57.25	60.50	62.50	59.44	61.20	0.76	0.592
viscera	168.41	167.67	160.23	164.35	163.23	160.35	35.39	0.708
Abdominal fat	85.00	84.25	80.34	82.75	80.47	83.23	3.97	0.474
Wings	2.76	2.73	2.79	2.55	2.79	2.55	5.28	0.951
Spleen	5.96^{c}	8.16^{b}	9.28^{a}	9.67^{a}	9.28^{a}	9.14^{a}	0.11	0.045
Bursa Fabricius	2.08	2.23	1.87	2.52	2.69	2.45	0.14	0.710
Gizzard fat	28.05	20.91	20.78	22.84	21.58	20.42	2.26	0.483

[1] Basal diet, [2] Basal diet + Vitamin E (200mg/kg), [3] Basal diet + *Zataria multiflora* (200 mg/kg), [4] Basal diet + *Zataria multiflora* (400 mg/kg), [5] Basal diet + *Ziziphora clinopodioides* (200 mg/kg), [6] Basal diet + *Ziziphora clinopodioides* (400 mg/kg), [7] Standard error of the mean.

Numbers with dissimilar letters in each row have a statistically significant difference ($P < 0.05$).

The thyme extracts had no significant effect on the relative carcass weight and internal organs in the starter (Table 5). However, they had a significant effect on some carcass traits, included body weight, breast, thigh, lung, and spleen. *Ziziphora clinopodioides* (400 mg/kg) showed the highest weight gain on the mentioned traits in the amount of 2503.53, 608.28, 42.35, and 6.44 g, respectively (grower). *Ziziphora clinopodioides* (400 mg/kg) on the body weight gain (2709.40), breast (1016.10), thigh (771.75 g), lung (63.14 g), liver (63.14 g), and spleen (9.67 g) had a highly significant effect in the finisher period.

The results of antioxidant properties showed in Table 8 and confirmed that the thyme extracts changed the amount of malondialdehyde and lipid peroxidation (Grower and Finisher) and also the levels of enzymes such as glutathione peroxidase (Grower and Finisher), and superoxide dismutase (Finisher). The dosage of 400 mg/kg *Zataria multiflora* essential oil showed a significant effect on glutathione peroxidase (0.35), superoxide dismutase (2.65), and lipid peroxidation (1.64) in the finisher period.

Table 8. The effect of different concentrations of thyme extracts and vitamin E on the lipid oxidation and antioxidant status of broilers in different growth periods

	Period	Treatments							
		Control[1]	VitE200[2]	ZN200[3]	ZM400[4]	ZC200[5]	ZC400[6]	*SEM*[7]	*P*-value
MDA[8] (nmol/mL)	Starter	1.19	0.91	1.02	0.82	0.90	0.81	0.20	0.215
	Grower	3.11^{a}	1.83^{c}	2.05^{b}	1.65^{d}	1.81^{c}	1.63^{d}	0.10	0.001
	Finisher	3.58^{a}	2.75^{b}	3.08^{ab}	2.48^{c}	2.71^{b}	2.45^{c}	0.10	0.001
GPx[9] (mmol/L)	Starter	0.07	0.10	0.09	0.11	0.07	0.10	0.05	0.365
	Grower	0.15^{b}	0.21^{ab}	0.19^{ab}	0.23^{a}	0.14^{b}	0.20^{a}	0.05	0.001
	Finisher	0.13^{d}	0.31^{a}	0.28^{ab}	0.35^{a}	0.21^{b}	0.10^{d}	0.12	0.001
SOD[10] (mmol/L)	Starter	0.61	0.86	0.80	0.88	0.74	0.80	0.07	0.425
	Grower	1.22	1.73	1.61	1.77	1.49	1.60	0.15	0.524
	Finisher	1.84^{c}	2.60^{a}	2.42^{ab}	2.65^{a}	2.23^{b}	2.40^{ab}	0.13	0.001
SGOT[11] (mmol/L)	Starter	0.10	0.09	0.08	0.09	0.07	0.09	0.03	0.362
	Grower	0.10	0.11	0.10	0.12	0.9	0.10	0.03	0.263
	Finisher	0.12	0.14	0.15	0.13	0.13	0.15	0.08	0.478
SGPT[12] (mmol/L)	Starter	0.07	0.09	0.07	0.08	0.08	0.07	0.02	0.489
	Grower	0.07	0.10	0.09	0.11	0.10	0.11	0.02	0.385
	Finisher	0.14	0.15	0.16	0.15	0.14	0.16	0.07	0.321
LP[13] (nmol/mL)	Starter	0.32	0.50	0.39	0.54	0.49	0.50	0.05	0.564
	Grower	0.64^{e}	1.01^{a}	0.78^{b}	1.09^{a}	0.98^{a}	1.12^{a}	0.01	0.001
	Finisher	0.97^{d}	1.52^{ab}	1.17^{c}	1.64^{a}	1.48^{b}	1.52^{ab}	0.10	0.05

[1] Basal diet, [2] Basal diet + Vitamin E (200mg/kg), [3] Basal diet + *Zataria multiflora* (200 mg/kg), [4] Basal diet + *Zataria multiflora* (400 mg/kg), [5] Basal diet + *Ziziphora clinopodioides* (200 mg/kg), [6] Basal diet + *Ziziphora clinopodioides* (400 mg/kg), [7] Standard error of the mean, [8] Malondialdehyde, [9] Glutathione peroxidase, [10] Superoxide dismutase, [11]Serum glutamate oxaloacetate transaminase, [12]Serum glutamate pyruvate transaminase, [13] Lipid peroxidation.
Numbers with dissimilar letters in each row have a statistically significant difference ($P < 0.05$).

Discussion

In this study, the results of feed intake were consistent with the other findings (Hoffman-Pennesi and Wu, 2010; Majeed *et al*., 2021). As broilers aged, thyme extract compounds increased feed intake and this might be through their effects on microbial populations and digestive processes. The presence of active ingredients in thyme, such as carvacrol, might have a stimulating effect on increasing the secretion of digestive leachate from the pancreas, liver, and intestines (Hashemipour *et al*., 2013). Furthermore, carvacrol and thymol in thyme extract increase lactic acid bacteria such as *lactobacilli* and *bifidobacteria* which can improve the immune system and animal growth (Baurhoo *et al*., 2007; Johny *et al*., 2010; El-Sayed and El-Sayed, 2020). In this study, high levels of carvacrol (39.94%) and thymol (32.92%) in *Zataria multiflora* could be the reason for the increase in the weight of broilers. However, increasing the amount of Menthone (19.47%) and Pulegone (23.06%) in *Ziziphora clinopodioides* extract due to its antioxidant, antibacterial, antiviral, anti-inflammatory, immune-boosting and LDL levels

caused weight gain (Thorup *et al*., 1983; Naderi *et al*., 2002; Oskoueian and Dalir 2019).

Consumption of thyme extracts increased weight gain in the periods of 0-24 and 0-42 days. However, at the age of 25-42 days, the most weight gain was achieved by consuming 400 mg/kg of *Ziziphora clinopodioides* essential oil. The active ingredients of thymol, carvacrol, cineole, alpha-pinene, menthol, and menthone are unique nutritional compounds due to their wide range of medicinal properties such as antioxidant, antibacterial, antiviral, anti-inflammatory, antidepressant, and immune system booster (Bento *et al*., 2013).

Improving the feed conversion ratio may be due to the presence of various chemical compounds in plant oil extracts, which will have beneficial effects on digestive activity and feed absorption as well as eliminate harmful factors such as available harmful microorganisms in the gastrointestinal tract (Platel and Srinivasan, 2004; Jamroz *et al*., 2006). On the other hand, the positive effect of thyme extract on feed consumption and average weight gain also affects the calculations of feed conversion ratio, and if one of the two factors is improved, the feed conversion ratio will also change. The use of *Ziziphora clinopodioides* (400 mg/kg) at the age of 25-42 and 0-42 days increased weight gain but the amount of feed intake among the treated groups was insignificant, so the feed conversion ratio of chickens improved significantly to 2.14 and 1.81 unite, respectively. Aromatic plants and plant extracts showed a significant increase in pancreatic lipase and amylase activity (Ramakrishna Rao *et al*., 2003) and may increase feed intake and weight gain due to increased food digestibility.

The means of energy and protein efficiency were not significant in 0-24 days. But, *Ziziphora clinopodioides* essential oil showed variation in mentioned efficiencies at the level of 200 and 400 mg/kg (25-42 and 0-42 days). Because the calculation of energy efficiency and protein is based on weight gain, so observing a significant difference in energy and protein efficiency is not unexpected.

Thyme extracts reduced albumin level and improved HDL-c value. Terpenoids in alcoholic extracts of medicinal plants significantly reduce the concentration of LDL-c (Fu *et al*., 2019; Sharma *et al*., 2021). A study found that carvacrol lowered plasma triglyceride levels, but did not affect plasma cholesterol (Lee *et al*., 2003). Serum triglyceride levels were reduced in the diets of laying hens using essential oils of thyme, rosemary, and sage, which were not parallel to the results of this study. The lowest amount of triglyceride was observed with the use of vitamin E (139.16 mg/dl) followed by 200 mg/kg of *Ziziphora clinopodioides* (145.79 mg/dl) (Bolukbasi, 2008). Aloum *et al*. (2020) showed that thymol and carvacrol at 150 ppm reduced cholesterol and triglycerides in the serum of Leghorn chickens and reducing in cholesterol may be due to differences in the chemical composition of thyme or the amount of dose tested. On the other hand, *Lactobacilli* can metabolize cholesterol in the small intestine and reduce its absorption through the bloodstream, thereby resulted in lowering blood cholesterol levels (Aloum *et al*., 2020). But in this study, the mean value of serum cholesterol did not show a significant difference with the use of different treatments.

The highest decrease in glucose level was recorded using *Ziziphora clinopodioides* (400 mg/kg) followed by the vitamin E group. Srinivasan (2005) reported that some plant species such as thyme, cinnamon, cloves, fragrant leaves, and turmeric could metabolize glucose due to their insulin-like factor. Lowering blood glucose levels lead to increase feed intake, and using thyme extract resulted in reducing blood glucose and increasing feed intake and body weight, which approved mentioned hypothesis. On the other hand, lowering glucose levels reduced cholesterol production. Glucose could affect pyruvate value and acetyl CoA, which is applied as a cholesterol precursor. Therefore, there was not enough acetyl CoA to synthesize serum cholesterol (Nelson *et al*., 2011).

Research on the properties of herbal products suggests that the use of herbal compounds and supplements stimulates lymphoid organs and hematopoietic cells (Al-Jaff, 2011; Rahimi *et al*., 2011). These plants have specific active ingredients to strengthen and proliferate fibroblasts of embryonic origin in chickens that are involved in the development of the immune system, lymphoid organs, and bone marrow tissue (Najafi and Torki, 2010; Paul *et al*., 2010; Tollba *et al*., 2010). Tollba *et al*. (2010) reported that various compounds in thyme could stimulate blood cell-producing organs due to their nutritional value (high levels of iron in thyme) and antioxidant effects.

Comparing the groups, vitamin E and *Ziziphora clinopodioides* at 400 mg/kg level showed the lowest hematocrit value. The low level of saponins increases the absorption of nutrients by increasing the diameter of the intestinal villi. The villi diameter leads to an increase in the intestinal permeability to molecules such as ferritin (Orczyk *et al*., 2020; Ulloa-Aguirre *et al*., 2021). Hernandez *et al*. (2004) revealed that feeding on thyme and cinnamon oil extracts in broilers significantly boosted hematocrit.

In poultry, uric acid is produced as the end product of nitrogen metabolism. Therefore, plasma and fecal uric acid levels can be an indicator of protein (amino acids) utilization. Also, total protein, uric acid, albumin, creatinine, and glucose can be considered as indicators of poultry liver damage (Mathur *et al*., 2001). *Zataria multiflora* (400 mg/kg) enhanced the amount of uric acid. Due to the

significant improvement in liver weight, the presence of some chemical compounds in thyme extract probably raised the weight and function of the liver. Thyme extract can affect the amount of protein consumed.

Glutathione peroxidase and superoxide dismutase enzymes are parts of the body's first defensive line against free radicals (Kryl'skii *et al*., 2019) and total antioxidant capacity is also an indicator of anti-radical activity, enzymatic and non-enzymatic antioxidants (Sen *et al*., 2010), therefore, it can be stated that the addition of thyme essential oil, as well as vitamin E, improved and strengthened the antioxidant status in broilers. The level of malondialdehyde as an indicator of lipid peroxidation in the body was reduced. The results were consistent with other researchers (Youdim and Deans, 2000; Hoffman-Pennesi and Wu, 2010; Roofchaee *et al*., 2011). Youdim and Deans (2000) reported that 42.5 (mg/kg/day) of thyme essential oil significantly increased the enzymatic activity of glutathione peroxidase, superoxide dismutase, and total antioxidant capacity in rat brains. Hoffman-Pennesi and Wu (2010) recorded that adding 0.2 mg/kg thymol and 2 and 4 mg/kg thyme essential oil to broiler chickens significantly increased serum antioxidant capacity. The results obtained from various studies using different quantities of thyme extract on the performance, blood parameters, and weight of organs in broilers were somewhat different that could be related to factors such as various concentrations of thyme extract due to growth environment, storage time, and condition as well as their quantity in the diet.

Conflict of Interest Statement

The authors declared that no conflict of interest exists.

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