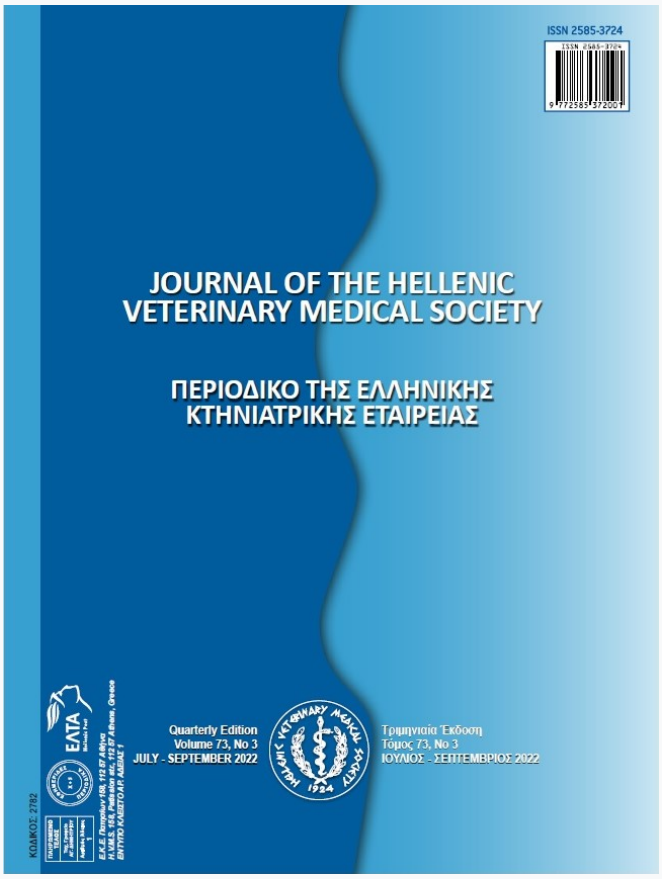
Journal of the Hellenic Veterinary Medical Society

Vol 73, No 3 (2022)



Effects of combined use of thyme powder and aqueous extract on growth performance, carcass and organ characteristics, blood parameters, enzymes, immune system and jejunum morphology in broilers

M Belali, A Seidavi, M Bouyeh

doi: 10.12681/jhvms.27469

To cite this article:

Belali, M., Seidavi, A., & Bouyeh, M. (2022). Effects of combined use of thyme powder and aqueous extract on growth performance, carcass and organ characteristics, blood parameters, enzymes, immune system and jejunum morphology in broilers. *Journal of the Hellenic Veterinary Medical Society*, *73*(3), 4465–4476. https://doi.org/10.12681/jhvms.27469

J HELLENIC VET MED SOC 2022, 73 (3): 4465- 4476
ΠΕΚΕ 2022, 73 (3): 4465- 4476

Research article
Ερευνητικό άρθρο

Effects of combined use of thyme powder and aqueous extract on growth performance, carcass and organ characteristics, blood parameters, enzymes, immune system and jejunum morphology in broilers

M. Belali[1], A.R. Seidavi[1*], M. Bouyeh[1]

[1]Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran

ABSTRACT: This experiment was performed to evaluate the effects of Thyme Extract (TE) and Thyme Powder (TP) on growth performance, carcass and organ characteristics, blood parameters, enzymes, immune system, and intestinal morphology in broilers. The experiment was performed based on a completely randomized design with 5 treatments, each with 4 replications and 10 Ross 308 male broilers in each replication for 42 days. Experimental treatments included aqueous extract of thyme (50 and 100 mg/kg feed) and thyme powder (150 and 250 mg/kg feed) which were used in combination with the basal diet (control). The effect of treatments was analyzed by SAS statistical software and the means were compared at 5% probability level with Duncan's multiple range test. The results showed that in the final period of the experiment, different levels of TE and TP had a significant effect on daily weight gain, feed intake and feed conversion ratio($P<0.05$) so that the desirable values were associated to treatment TE (100) + TP (250). Different levels of TE and TP significantly affected European index, economic value and final weight ($P<0.05$), with the highest means observed in TE (100) + TP (250), and the lowest in TE (0) and TP (0) group. Different levels of TE and TP had significant effects on the relative weight of thymus, live weight, wing weight, abdominal fat and pancreas ($P<0.05$). The effect of different levels of TE and TP on the immunity was significant throughout the experimental period ($P<0.05$). The higher levels of TE and TP increased the villi length, villi width, crypt depth, layer thickness and the ratio of villi length to crypt depth compared to the control treatment. Based on the results of the present study, the use of TE (100 mg/kg) + TP (250 mg/kg) in the diet is recommended as a dietary supplement in Ross 308 broilers.

Keywords: Feed additive; *Thymus vulgaris*; chicks; growth; antibody; cholesterol; intestinal morphology

Corresponding Author:
A.R. Seidavi, Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran
E-mail address: alirezaseidavi@iaurasht.ac.ir

Date of initial submission: 09-07-2021
Date of acceptance: 26-10-2021

INTRODUCTION

For many years, the use of feed additives in poultry diet has been considered as a mean of increasing the bird's productivity. Antibiotics are among the food additives that are used to prevent the growth of intestinal pathogens, stimulate the growth of birds and improve the performance of poultry feed. After several years, it was concluded that the development of resistance to pathogens, as well as the possibility of the presence of antibiotic residues in manufactured products, are among the parameters that have limited their use in animal and poultry feed. Moreover, in European countries, the use of antibiotics in the poultry industry is prohibited due to the possibility of creating resistant strains, and in other countries, their use is limited (Madrid et al., 2003).

Nowadays, herbal feed additives such as essential oils or plant extracts (phytogenic compounds) have attracted a lot of attention as an alternative to dietary antimicrobial agents. There is evidence that essential oils stimulate the secretion of digestive enzymes, balance intestinal microbial ecosystems and thus improve performance in chickens (Williams, 2001)

Hernandez et al. (2004) showed that some plant extracts such as thyme cause faster growth and improve intestinal digestion, starch digestibility, dry matter utilization of diets and carcass traits in broilers. Thyme (*Thymus vulgaris* L.) is a herbaceous, aromatic plant belonging to the mint family. Thymol and carvacrol are its important active ingredients, but other substances such as paracetamol, linalool and cineole, flavonoids, terpenes, spicy compounds and a number of other active ingredients are also found in thyme (Sharififar et al., 2007). Addition of thyme essential oil to the diet or drinking water of broilers has led to weight gain and improved feed conversion ratio (Alcicek et al., 2004). Cross et al. (2007) also reported that thyme oil has a positive effect on the performance of broilers.

(Al-Kassie 2009) investigated the effect of thyme and cinnamon extracts at 100 and 200 mg/kg diet levels in broilers and reported that growth performance traits (daily weight gain, feed intake and feed conversion ratio) in birds that received these two extracts were significantly improved; higher levels of these two extracts showed better results than lower levels.

The most important phenolic compounds in thyme are thymol and carvacrol. The antibacterial, antifungal and anti-coccidiotic properties of thyme have been attributed to these compounds. The presence of beneficial microflora has been shown to increase villi length, crypt and intestinal cell proliferation, but pathogenic bacteria produce toxic compounds such as ammonia, destroying the epithelial layer and increasing cell transformation to regenerate atrophic cells. As a results, villi height decreases and intestinal crypt depth increases (Bakkali et al., 2008). Thyme causes the secretion of digestive enzymes such as amylase and chymotrypsin and increases the amount of feed intake (Denli et al., 2004).

This study was implemented to clarify the contradictory and incomplete results regarding the effect of antioxidant compounds on the aforementioned factors, and the limited results regarding the simultaneous evaluation of the effects of thyme powder and its extract on growth performance, carcass and gastrointestinal characteristics, blood parameters, immune system, and intestinal morphology in broiler chickens.

MATERIALS AND METHODS

This study was conducted in one of the broiler farms located in Masal, Iran. The 200 one-day chicks (45±2 g) was allocated into 5 treatments, each with 4 replications and 10 chickens per replication for 42 days. Two levels of thyme powder (150 and 250 mg/kg) and two levels of aqueous thyme extract (at levels of 50 and 100 mg/kg) in the diet for all 42 days of rearing, were applied as follows. The treatments were

Treatment 1: TE (0) + TP (0), aqueous extract of thyme (0 mg/kg) + thyme powder (0 mg/kg)

Treatment 2: TE (50) + TP (150), aqueous extract of thyme (50 mg/kg) + thyme powder (150 mg/kg)

Treatment 3: TE (50) + TP (250), aqueous extract of thyme (50 mg/kg) + thyme powder (250 mg/kg)

Treatment 4: TE (100) + TP (150), aqueous extract of thyme (100 mg/kg) + thyme powder (150 mg/kg)

Treatment 5: TE (100) + TP (250), aqueous extract of thyme (100 mg/kg) + thyme powder (250 mg/kg)

which was used in combination with the basal diet

The aqueous extract of thyme and thyme powder were produced by Zarghani Pharmaceutical Company (Sabzevar, Iran). Diets were adjusted according to the poultry nutritional requirements table containing the minimum nutrients recommended in the Ross 308 strain feeding guide Manual (Table 1). Chickens were

reared in 1 × 1 m cages on a cellulose roll bed for 42 days. Each cage had a single drinker and feeder. The temperature of the breeding hall decreased to 33 °C in the first days and then gradually to 23 °C on the 18th day of breeding and then continued until the end of the period. Environmental conditions were similar for all groups (20 pens) and included 23 hours of light exposure and one hour of darkness. The humidity of the hall was 65 to 70%. Access to water and feed was similar and free during the rearing period. In addition, the birds were vaccinated against infectious bronchitis (10th day of age), Newcastle (4th, 21st and 35th days of age) and Infectious Bursal disease (12th day of age) (NDV; Viscerotropicvelogenic strain). All vaccines were obtained from Razi Serum and Vaccine Institute (Karaj, Iran).

Economic growth performance and returns

The weight gains of chickens per pen in periods of 1 to 10, 11 to 24 and 25 to 42 days were measured on a digital balance with an accuracy of 0.01 g. At the end of each period (initial 1 to 10, growth 11 to 24 and final 25 to 42) the amount of feed left was weighed and subtracted from the amount of feed given at the beginning of each period, to calculate the amount of feed consumed. Feed conversion ratio was calculated by dividing feed intake by weight gain for days 1 to 10, 11 to 24, 25 to 42 and the whole period. The following formula reported by Ghoreyshi et al. (2019) was used to calculate the European production index:

European production index: Average live weight (g) × Retention rate/Food conversion ratio × Number of breeding days × 10

The following formula was used to measure the cost of feed per kilogram of live chicken. The daily price of thyme powder and thyme aqueous extract used was calculated separately for each diet and placed in the formula.

Cost of feed per kilogram of live chicken = (weight of a chicken at 42 days in kilograms/feed price during 42 days for each chicken in Rials

Characteristics of carcasses and digestive organs

At the end of the experiment, after two hours of starvation, 2 birds were slaughtered from each replicate, weighing close to the average, and stuffed carcass weight, full carcass weight, empty carcass weight, breast weight, thigh weight, wing weight as well as the weight of internal organs (pancreas, heart, gills, spleen, bursa of Fabricius, liver, abdominal fat,

Table 1. Ingredients, chemical composition, and energy of the used diets (from 1 to 42 d of age)

Ingredients (g/kg as-fed)	Starter diet (1st-10th days of age)	Grower diet (11st-24th days of age)	Finisher diet (25th-42nd days of age)
Corn	47.03	59.60	65.99
Wheat	5.58	5.00	5.00
Soybean meal (44% Crude protein)	29.02	16.15	10.28
Corn gluten	10.00	11.48	11.50
soy oil	3.50	3.40	3.09
Limestone	1.45	1.23	1.00
Di-calcium phosphate	1.95	1.80	1.83
Salt	0.20	0.20	0.20
Vitamin and mineral supplements[1]	0.50	0.50	0.50
DL-methionine	0.52	0.58	0.57
L-lysine hydrochloride	0.25	0.06	0.04
Calculated compounds			
Metabolizable energy (kcal/kg)	2950	3000	3050
Crude protein (%)	22	20	19
Lysine (%)	1.3	1.2	1.1
Methionine (%)	0.56	0.54	0.52
Met+Cys (%)	0.92	0.90	0.88
Calcium (%)	1.04	0.95	0.92
Available phosphorus	0.52	0.47	0.41

1. The amount of vitamins and minerals per kg of the final diet:vitamin A, 9000 IU; vitamin D3, 3000 IU; vitamin E, 18 IU; vitamin K3, 3 mg; vitamin B1(Thiamine), 1/8 mg;vitamin B2(Riboflavin),6 mg; vitamin B6(Pyridoxine),3 mg; vitamin B12(Cyanocobalamin), 0/012 mg;vitamin B3(Niacin),30 mg; vitamin B9(Folic acid),1 mg;vitamin H3(Biotin),0/24mg;vitamin B5(Pantothenic acid),10 mg; 500 mg;Choline,100 mg;Mn,100 mg;Zinc,80 mg;Iron,10 mg; Cu,1 mg;I,0/2 mg;Selenio

Table 2. Growth performance mean (±SEM) of Ross 308 broilers at starter, grower, finisher and whole periods of age fed diets containing the different levels of thyme extract and thyme powder from 1st-42nd days of age

	1st-10th days of age			11st-24th days of age			25th-42nd days of age			1st-42nd days of age		
	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed conversion ratio	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed conversion ratio	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed conversion ratio	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed conversion ratio
Thyme extract (0 mg/kg)-thyme powder (0 mg/kg)	18.10	9.65^{c}	1.88	48.40^{c}	32.71^{b}	1.48	144.83^{c}	68.57^{c}	2.12	82.52^{c}	42.59^{c}	1.94^{a}
Thyme extract (50 mg/kg)-thyme powder (150 mg/kg)	21.35	12.65^{ab}	1.69	61.06^{b}	49.07^{a}	1.27	165.58^{ab}	85.83^{ab}	1.92	96.40^{a}	56.15^{a}	1.72^{b}
Thyme extract (50 mg/kg)-thyme powder (250 mg/kg)	18.17	11.35^{bc}	1.61	57.35^{b}	48.81^{a}	1.18	148.35^{bc}	71.87^{c}	2.07	87.02^{bc}	49.77^{b}	1.75^{b}
Thyme extract (100 mg/kg)-thyme powder (150 mg/kg)	22.20	13.75^{a}	1.62	70.24^{a}	52.32^{a}	1.36	152.05^{bc}	77.00^{bc}	1.98	93.87^{ab}	53.71^{ab}	1.75^{b}
Thyme extract (100 mg/kg)-thyme powder (250 mg/kg)	19.97	12.40^{ab}	1.61	61.97^{ab}	50.25^{a}	1.24	173.16^{a}	88.58^{a}	1.96	99.63^{a}	57.67^{a}	1.73^{b}
P-value	0.06	0.006	0.08	0.001	0.0004	0.26	0.02	0.005	0.12	0.002	<0.0001	<0.0001
SEM	1.10	0.66	0.07	2.75	2.53	0.10	5.87	3.58	0.05	2.55	1.59	0.02

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter do not differ significantly (P>0.05); SEM: Standard Error of Means

weight of duodenum, jejunum and ileum) were measured as also described by Zahirian et al., (2019).

Blood serum parameters and digestive enzymes

At the end of the experiment (after 42 days), 2 birds weighing close to the average were randomly selected and blood samples of 5 ml were collected from the wing vein. The samples were centrifuged at room temperature at 5000 rpm for 3 min (5702, Eppendorf, Germany) and the serum was separated and transferred to microtubes and transferred to the laboratory. The serum was stored at -20 °C until blood metabolites were measured. Serum was thawed at room temperature and glucose, triglyceride, cholesterol, total protein, albumin, globulin, creatine kinase, lactate dehydrogenase, VLDL (High-density lipoprotein), HDL (high-density lipoprotein), LDL (Low-density lipoprotein) alanine transferase and alkaline phosphatase were measured. These parameters were measured with Pars test kits and by autoanalyzer (Hitachi brand model 917/Japan) based on Gholami et al. (2020).

Immune response

To evaluate humoral safety, broilers were immunized against sheep red blood cells (SRBC). To prepare an SRBC injection suspension, blood samples were taken from 3 sheep and poured into jars containing EDTA. The blood cells were washed three times in PBS saline phosphate buffer, and a suspension of 2% SRBC in PBS was prepared. All the above steps were performed under sterile conditions. Then it was injected into the vein of the birds 7 and 14 days after the first and second injections and blood samples were taken on days 35 and 42. SRBC was measured by the hemagglutination method. To measure the antibody titer, plates for microhemagglutination were prepared, which had 96 V-shaped wells in 12 columns (1 to 12) and 8 rows (A to H). The Van derzipp method was used to measure total antibody titer. According to this method for measuring total anti-SRBC, 50 µl of the serum sample was mixed with 50 µl saline phosphate buffer (PBS) inside the microtiter plate and then 50% of 2% SRBC suspension was added to each well and placed at room temperature for 4 to 5 hours. Titers were expressed based on $\log_2$. The highest rate of complete agglutination was expressed (Pourhossein et al., 2015). In addition, the birds were vaccinated against Newcastle disease and influenza (NDV; Viscerotropicvelogenic strain). All vaccines were obtained from Razi Serum and Vaccine Institute (Karaj, Iran (Table 2). NDV). Influenza blood samples were taken from 2 birds per pen on 28 and 42 days and then a hemagglutination inhibition (HI) test according to OIE standard was performed on Newcastle and influenza serum titers, first 25 µl PBS It was poured into all wells, then 25 microliters of bird serum was diluted in the first well and diluted to the last well. The mechanical shaker was stored and the microplate was placed at 25 ° C for 30 minutes, then 25 µl of 1% red blood cells was added to all the slides and the microplate was placed on the mechanical shaker again for 15 seconds. The microplate was then placed at 25 ° C for 30 minutes and the results were recorded. It was used for HI test. The titres were diluted based on $\log_2$. The red blood cells used were also obtained from SPF chickens. On day 42, for the total white blood cell count and their differential count, 2 birds were collected from each pen and their blood was transferred to tubes containing anticoagulant. Blood cells were determined by cell staining, differentiation and visual counting under a light microscope (Golrokh et al., 2015).

Table 3. Economical performance mean (±SEM) of Ross 308 broilers at 42nd days of age fed diets containing the different levels of thyme extract and thyme powder from 1st-42nd days of age

	Weight of 1 chick at 42nd days of age (gr/chick)	Feed cost per kg live weight (Rial/kg)	European production index
Thyme extract (0 mg/kg)-thyme powder (0 mg/kg)	1828.75^{c}	52978.30^{a}	224.90^{c}
Thyme extract (50 mg/kg)-thyme powder (150 mg/kg)	2398.50^{a}	48006.60^{b}	333.30^{a}
Thyme extract (50 mg/kg)-thyme powder (250 mg/kg)	2130.60^{b}	48909.30^{b}	290.50^{b}
Thyme extract (100 mg/kg)-thyme powder (150 mg/kg)	2296.00^{ab}	49405.80^{b}	312.37^{ab}
Thyme extract (100 mg/kg)-thyme powder (250 mg/kg)	2462.10^{a}	48952.90^{b}	339.28^{a}
P-value	<0.0001	0.0007	<0.0001
SEM	66.83	643.23	11.57

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter do not differ significantly (P>0.05); SEM: Standard Error of Means

Intestinal morphology

At 42 days of age, immediately after slaughter, tissue sections of the small intestine (1 cm from the middle part of the intestinal jejunum) were cut and placed immediately in cans containing 10% formalin. After three steps of formalin replacement and stabilization of tissue samples, 5 mm sections were cut with a sterile surgical blade and the tissue sections were prepared by the hematoxylin and eosin (Hand E) method. Xylol (10 min), 96% alcohol (5 min), 100% alcohol (5 min) and hematoxylin (10 min), once inserted into an alcohol container and then, three steps of rinsing with distilled water, eosin (3 min), 96% and 100% alcohol were placed in containers and finally clarification was performed in two xylol containers, and then the slides were placed under a microscope with a graduated lens and the villi height and crypt depth were examined.

Histomorphometric indices were studied using hematoxylin and eosin staining of the jejunum, which were the total thickness of the intestinal wall from the base of the villi to the serous layer, the length of the villi, the thickness of the villi, the ratio of the length of the villi to the depth of the crypts, and the thickness of the epithelium.

Statistical Analysis

The obtained data were statistically analyzed using analysis of variance by SAS statistical software. The mean of treatments was compared at the 5% probability level with Duncan's multiple range test. The design used in this experiment was completely random.

RESULTS AND DISCUSSION

Growth performance

The results of using different levels of thyme aqueous extract and thyme powder on the performance of broilers are presented in Tables 2 and 3. In the period of 1 to 10 days, different levels of TE and TP did not cause significantly different feed intake or conversion ratio (P>0.05), but there was a significant difference in daily weight gain (P<0.05); the highest mean was for treatment TE (100) +TP (150) and the lowest mean was for TE (0) + TP (0). In the periods of 11 to 24 and 25 to 42 days, the levels of TE and TP did not significantly affect the improving the conversion ratio (P>0.05), but there was a significant difference in daily weight gain and feed intake (P<0.05). In the period of 1 to 42 days, the levels of TE and TP made a significant difference on daily weight gain, feed intake and conversion ratio improvement (P<0.05); the best treatment was TE (100) + TP (250); the weakest performing treatment was TE (0) + TP (0), the control. Also, the effect of the different levels of aqueous extract of thyme and thyme powder significantly influenced the European index, economic value and total weight (P<0.05).

Our growth parameters was low and performance objectives of the Ross 308 were not met since the chicks were fron a aged breeder floch and also there is a coccidiose infection in early period of experiment. The effect of deamination activity of microbes and their increased decomposition rate are due to the secretion of substances such as microbial urease. Since the use of medicinal plants reduces the microbial population of the gastrointestinal tract, the rate of decomposition of proteins and amino acids in the digestive system decreased and more of them were absorbed, resulting in improved feed conversion ratio (Lee et al., 2003b). Beneficial antioxidant compounds in medicinal plants also improve nutrient uptake by protecting intestinal villi, thereby improving bird performance, which is consistent with the results of the present experiment (Manzanilla et al., 2001).

In one experiment, chickens that received alcoholic thyme extract in drinking water had the highest weight gain (Abdulkarimi et al., 2011), which is consistent with the results of the present experiment. This result may be related to antimicrobial and stimulant properties of thyme extract due to its low pH. Manzanilla et al. (2001) also reported that the beneficial antioxidant compounds of medicinal plants protect intestinal villi, improve nutrient absorption and thus improve bird performance.

Kalantar et al. (2011) also used thyme essential oil in drinking water and at the end of the experiment the best conversion ratio was observed at the rate of 0.2%, which is consistent with the results of the present experiment. Rahimi et al. (2011) reported that supplementing the diet of broilers with 0.1% thyme extract improved feed conversion ratio compared to the control group. On the contrary, Okac et al. (2008) added dried thyme powder and thyme essential oil to the diet of broilers but did not observe a significant difference in feed intake in any of the experimental periods; this result differs from that obtained in the present experiment.

Characteristics of carcasses and some gastrointestinal organs

The effects of experimental treatments on carcass characteristics and digestive organs are shown in Tables 4 and 5. The results showed that the use of different levels of TE and TP had a significant effect on the defeather body weight, full and empty abdomen carcass weights, eviscerated carcass, and relative weight of wings and abdominal fat (P<0.05). Abazari et al.

Table 4. Mean (±SEM) of economically relevant carcass characteristics of Ross 308 broilers at 42nd days of age fed diets containing the different levels of thyme extract and thyme powder from 1st-42nd days of age

	Live body weight (gr)	Defeather body weight (gr)	Full abdomen carcass weight (gr)	Empty abdomen carcass weight (gr)	Eviscerated carcass (%)	Relative weight of crop (%)	Relative weight of breast (%)	Relative weight of drumsticks (thighs) (%)	Relative weight of wings (%)	Relative weight of abdominal fat (%)	Relative weight of pancreas (%)
Thyme extract (0 mg/kg)-thyme powder (0 mg/kg)	2267.50^{c}	2031.80^{c}	1869.30^{c}	1596.50^{c}	78.53^{b}	0.44	26.93	21.99	8.27^{a}	1.65^{a}	0.31
Thyme extract (50 mg/kg)-thyme powder (150 mg/kg)	2755.00^{ab}	2565.00^{ab}	2425.00^{ab}	2152.50^{ab}	83.79^{a}	0.83	29.54	21.55	6.02^{b}	0.68^{b}	0.26
Thyme extract (50 mg/kg)-thyme powder (250 mg/kg)	2530.00^{bc}	2330.00^{bc}	2178.80^{bc}	1952.50^{bc}	83.77^{a}	0.82	32.34	23.46	6.10^{b}	0.52^{b}	0.20
Thyme extract (100 mg/kg)-thyme powder (150 mg/kg)	2635.00^{abc}	2451.30^{ab}	2313.80^{ab}	2063.80^{ab}	84.06^{a}	1.19	29.54	20.90	5.76^{b}	0.64^{b}	0.20
Thyme extract (100 mg/kg)-thyme powder (250 mg/kg)	3000.00^{a}	2798.80^{a}	2637.50^{a}	2385.00^{a}	85.13^{a}	0.83	31.32	20.97	5.43^{b}	0.50^{b}	0.17
P-value	0.02	0.01	0.008	0.006	<0.0001	0.43	0.07	0.33	0.001	0.0003	0.15
SEM	130.75	131.15	125.94	123.30	0.63	0.26	1.26	0.93	0.39	0.15	0.04

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter do not differ significantly (P>0.05); SEM: Standard Error of Means

Table 5. Mean (±SEM) of organ characteristics of Ross 308 broilers at 42nd days of age fed diets containing the different levels of thyme extract and thyme powder from 1st-42nd days of age

	Relative weight of gizzard (ventriculus) (%)	Relative weight of heart (%)	Relative weight of liver (%)	Relative weight of proventriculus (%)
Thyme extract (0 mg/kg)-thyme powder (0 mg/kg)	2.18	0.75	2.56	0.48
Thyme extract (50 mg/kg)-thyme powder (150 mg/kg)	2.52	0.52	2.47	0.48
Thyme extract (50 mg/kg)-thyme powder (250 mg/kg)	2.68	0.62	2.43	0.46
Thyme extract (100 mg/kg)-thyme powder (150 mg/kg)	2.93	0.55	2.87	0.47
Thyme extract (100 mg/kg)-thyme powder (250 mg/kg)	2.42	0.53	2.22	0.41
P-value	0.10	0.19	0.41	0.76
SEM	0.18	0.07	0.23	0.04

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter do not differ significantly (P>0.05); SEM: Standard Error of Means

(2011) and Rahimi et al. (2011) used mixtures of plant essential oils and did not observe a significant effect on the relative weight and carcass components, which is consistent with the results of the present experiment. The effect of alcoholic thyme extract supplement on growth performance and some carcass traits was investigated and it was reported that the relative wing weight of chickens that received a level of 4% was significantly higher than for chickens that did not received thyme. Thyme extract in drinking water significantly increased the relative weight of breast and wings. Abdulkarimi et al. (2011) concluded that alcoholic thyme extract in drinking water improves the performance and relative weight of broiler chickens, which does not correspond to the results obtained from the present experiment.

Belali et al., (2021) found the thyme can improve the fatty acid profile of breast meat in broiler chicks. Pournazari et al., (2017) investigated the effect of thyme, prebiotics and probiotics on carcass traits in broilers. The results of this experiment showed that the use of thyme essential oil caused a relative reduction in thigh and wing weight, which is consistent with the results of the present experiment.

Intestinal parts

The effects of experimental treatments on different parts of the intestine are shown in Table 6. Different levels of TE and TP had a significant effect on the weight ratio of jejunum and colon ($P<0.05$); the highest mean weight ratio level was for TE (0) + TP (0). Denli et al. (2004) reported that a mixture of thyme and peppermint increased the relative weight of the ileum compared to the control; this did not match the results of the present experiment.

Blood parameters and liver enzymes

Data on the effect of TE and TP on blood parameters are shown in Table 7. The results showed that

Table 6. Mean (±SEM) of intestinal segments of Ross 308 broilers at 42nd days of age fed diets containing the different levels of thyme extract and thyme powder from 1st-42nd days of age

	Relative weight of rectum (%)	Relative weight of duodenum (%)	Relative weight of jejunum (%)	Relative weight of ileum (%)	Relative weight of colon (%)	Relative weight of right cecum (%)	Relative weight of left cecum (%)
Thyme extract (0 mg/kg)-thyme powder (0 mg/kg)	0.24	0.72	1.29^{a}	0.54	0.38^{a}	0.19	0.19
Thyme extract (50 mg/kg)-thyme powder (150 mg/kg)	0.19	0.65	0.97^{bc}	0.57	0.30^{bc}	0.17	0.17
Thyme extract (50 mg/kg)-thyme powder (250 mg/kg)	0.19	0.73	1.14^{ab}	0.60	0.35^{ab}	0.17	0.17
Thyme extract (100 mg/kg)-thyme powder (150 mg/kg)	0.19	0.66	0.95^{bc}	0.55	0.29^{bc}	0.16	0.15
Thyme extract (100 mg/kg)-thyme powder (250 mg/kg)	0.15	0.75	0.83^{c}	0.53	0.27^{c}	0.15	0.15
P-value	0.07	0.91	0.006	0.83	0.03	0.15	0.09
SEM	0.02	0.09	0.08	0.05	0.02	0.01	0.01

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter do not differ significantly ($P>0.05$); SEM: Standard Error of Means

Table 7. Blood constitutes mean (±SEM) of Ross 308 broilers at 42nd days of age fed diets containing the different levels of thyme extract and thyme powder from 1st-42nd days of age

	Cholesterol (mg/dl)	Triglycerides (mg/dl)	VLDL (Very low density lipoprotein) (mg/dl)	HDL Cholesterol (High Density Lipoproteins) (mg/dl)	LDL Cholesterol (Low Density Lipoproteins) (mg/dl)	LDL /HDL	Glucose (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Thyme extract (0 mg/kg)-thyme powder (0 mg/kg)	131.25	76.25	15.27	72.25	36.00	0.50	197.75	3.80	2.47	1.32
Thyme extract (50 mg/kg)-thyme powder (150 mg/kg)	137.75	90.75	18.15	79.25	34.50	0.43	163.50	3.30	1.80	1.50
Thyme extract (50 mg/kg)-thyme powder (250 mg/kg)	155.00	77.50	15.50	81.50	43.00	0.53	145.00	4.10	2.50	1.60
Thyme extract (100 mg/kg)-thyme powder (150 mg/kg)	148.75	140.75	28.15	80.25	38.50	0.47	179.25	3.95	2.22	1.72
Thyme extract (100 mg/kg)-thyme powder (250 mg/kg)	148.75	56.25	11.25	83.75	41.50	0.49	138.00	3.75	2.25	1.50
P-value	0.34	0.76	0.76	0.58	0.50	0.23	0.18	0.45	0.71	0.84
SEM	8.62	46.60	9.32	5.04	3.83	0.03	18.34	0.31	0.38	0.25

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter do not differ significantly ($P>0.05$); SEM: Standard Error of Means

the effects of different levels of thyme aqueous extract and thyme powder on the blood parameters of broilers were not significant (P>0.05). Tymorizade et al. (2010) reported a significant decrease in blood cholesterol and triglyceride levels in experiments on broiler chickens using thyme extract, which did not match the results of the present experiment.

(Demir et al. 2003) studied the effect of powder of several medicinal plants (garlic, thyme, cinnamon and oregano) on the hematological values of broilers and reported that these extracts did not have a significant effect on the concentration of plasma triglycerides of broilers; that is consistent with the results of the present experiment.

The effect of different levels of TE and TP on liver enzymes is shown in Table 8. The results showed that the effects of different levels of TE and TP on alkaline phosphatase, alanine transaminase, lactate dehydrogenase and creatine kinase of broilers in the whole period were not significant (P>0.05). The antioxidant properties of thyme seem to reduce the destructive oxidative effect of the toxin on the liver and reduce cholesterol, triglycerides and liver enzymes due to the inhibitory effect of these extracts on key enzymes such as HMG-COA reductase. They have also been implicated in lipid and cholesterol production (Sarica et al., 2005).

In a study by Aiatollahi et al. (2016), all three enzymes LDH, SGPT and SGOT showed a significant decrease with the addition of thyme and an increase in the percentage of thyme compared to the control group, which does not correspond to the results obtained from the present experiment.

Immune Response

The effect of different levels of TE and TP on the function of the humoral immuity including antibody against sheep red blood cell, antibody against sheep red blood cell, antibody against avian influenza (42nd day of age), antibody against avian influenza (28th day of age), antibody against Newcastle dis-

Table 8. Liver enzymes mean (±SEM) of Ross 308 broilers at 42nd days of age fed diets containing the different levels of thyme extract and thyme powder from 1st-42nd days of age

	Alkaline phosphatase (U/L)	Alanine transaminase (IU/L)	Lactate dehydrogenase (IU/L)	Creatine kinase (IU/L)
Thyme extract (0 mg/kg)-thyme powder (0 mg/kg)	5048.30	239.25	4280.00	10051.00
Thyme extract (50 mg/kg)-thyme powder (150 mg/kg)	4580.30	278.25	4673.00	11550.00
Thyme extract (50 mg/kg)-thyme powder (250 mg/kg)	3717.00	341.25	6017.00	16601.00
Thyme extract (100 mg/kg)-thyme powder (150 mg/kg)	2976.80	414.75	5486.00	27542.00
Thyme extract (100 mg/kg)-thyme powder (250 mg/kg)	3974.30	372.50	6164.00	29999.00
P-value	0.23	0.22	0.64	0.13
SEM	637.16	55.41	1028.47	6307.77

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter do not differ significantly (P>0.05); SEM: Standard Error of Means

Table 9. Immune response mean (±SEM) of Ross 308 broilers fed diets containing the different levels of thyme extract and thyme powder from 1st-42nd days of age

	White blood cells ($n \times 10^3$/mL)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Antibody against Newcastle disease (28 days) (lg 2)	Antibody against Newcastle disease (42 days) (lg 2)	Antibody against avian influenza (28 days) (lg 2)	Antibody against avian influenza (42 days) (lg 2)	Antibody against sheep red blood cell (35 days)	Antibody against sheep red blood cell (42 days)
Thyme extract (0 mg/kg)-thyme powder (0 mg/kg)	5250.00	14.25^{b}	80.00^{a}	5.50	4.00	6.00	2.50^{ab}	4.50^{b}	5.50^{a}	6.75^{b}
Thyme extract (50 mg/kg)-thyme powder (150 mg/kg)	2550.00	39.50^{a}	52.00^{bc}	8.75	2.25	5.25	2.25^{b}	4.25^{b}	4.25^{b}	6.25^{b}
Thyme extract (50 mg/kg)-thyme powder (250 mg/kg)	4400.00	24.75^{ab}	71.50^{a}	3.75	2.50	6.25	1.25^{c}	3.25^{b}	3.00^{c}	5.00^{c}
Thyme extract (100 mg/kg)-thyme powder (150 mg/kg)	1450.00	25.50^{ab}	69.00^{ab}	5.50	2.75	5.50	3.25^{a}	6.25^{a}	6.25^{a}	8.00^{a}
Thyme extract (100 mg/kg)-thyme powder (250 mg/kg)	2300.00	41.25^{a}	49.50^{c}	8.50	1.25	4.50	1.25^{c}	4.50^{b}	4.00^{bc}	7.75^{a}
P-value	0.31	0.03	0.007	0.25	0.07	0.22	0.002	0.002	0.0002	<0.0001
SEM	1378.22	5.85	5.66	1.76	0.59	0.54	0.32	0.41	0.38	0.32

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter do not differ significantly (P>0.05); SEM: Standard Error of Means

ease (28th day of age), antibody against Newcastle disease (42nd day of age), eosinophils, lymphocytes, neutrophils, white blood cells are shown in Table 9. The results showed that the effects of different levels of TE and TP on the all above immune system function of broilers throughout the period were significant (P<0.05) except antibody against Newcastle disease (28th day of age), antibody against Newcastle disease (42nd day of age), eosinophils and white blood cells.

Almremdhy and Al-khafaji MA (2020) found positive effects of drinking thyme extract on immune response of broiler chickens. The effects of experimental treatments on immunity related organs are shown in Table 10. The results showed that the use of different levels of TE and TP had a significant effect on the relative weight of thymus (P<0.05). It has been reported that the feeding of medicinal plants stimulates the growth of immune organs of broilers and causes a significant increase in their weight (Souri et al., 2015; Behboudi et al., 2016; Rafat Khafar et al, 2019, Ahmadian et al., 2020). The presence of bioactive compounds in thyme probably stimulates cell proliferation in these organs. The bursa, thymus and spleen are among the organs of the immune system, and improving the weight of each of them can improve the condition of the bird's immune system. Perhaps the higher relative weight in the bursa and thymus indicates the effect of thyme extract on the bird's immune status.

Beheshti et al. (2010) reported that 2% of a mixture of thyme, mint and savory in the diets of laying hens improved the performance of blood parameters and immunity, which is consistent with the results of the present experiment.

Rafiee et al. (2013) investigated the effect of thyme and ginger extract on the safety of broilers. In this experiment, alcoholic thyme extract with a concentration of 0.5% was added to the diet and blood samples were taken at 42 days of age. The results showed that the use of TE increases the specific antibody titer of Newcastle disease vaccine, which is consistent with the results obtained from the present experiment.

Silymarin Phytosomes in the seeds of St. John's

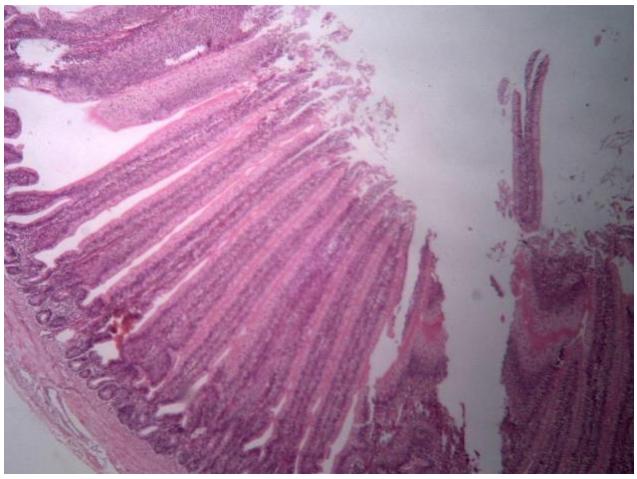
Table 10. Immunity related organ mean (±SEM) of Ross 308 broilers at 42nd days of age fed diets containing the different levels of thyme extract and thyme powder from 1st-42nd days of age

	Relative weight of thymus (%)	Relative weight of spleen (%)	Relative weight of bursa of fabricius (%)
Thyme extract (0 mg/kg)-thyme powder (0 mg/kg)	0.48[a]	0.13	0.19
Thyme extract (50 mg/kg)-thyme powder (150 mg/kg)	0.33[b]	0.10	0.13
Thyme extract (50 mg/kg)-thyme powder (250 mg/kg)	0.34[b]	0.11	0.17
Thyme extract (100 mg/kg)-thyme powder (150 mg/kg)	0.34[b]	0.10	0.15
Thyme extract (100 mg/kg)-thyme powder (250 mg/kg)	0.33[b]	0.10	0.17
P-value	<0.0001	0.55	0.08
SEM	0.01	0.01	0.01

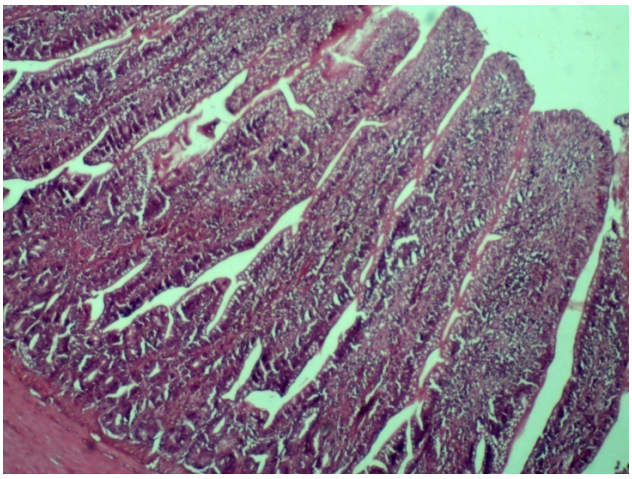
[*] Means within each column of dietary treatments with no superscript letter or at least one common superscript letter do not differ significantly (P>0.05); SEM: Standard Error of Means

Table 11. Profile of breast fatty acids Ross 308 broilers in 42-day diets containing different levels of thyme extract and thyme powder from 1st-42nd days of age

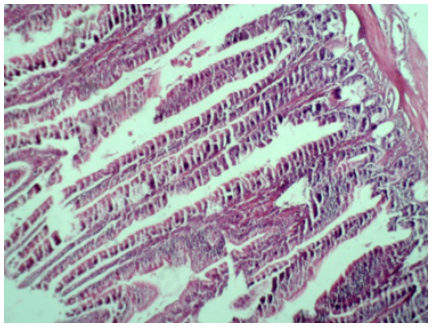
	The length of the villi	The width of the villi	Crypt depth	Layer thickness	The length of the villi/crypt depth
Thyme extract (0 mg/kg)-thyme powder (0 mg/kg)	995.00	151.57	142.57	159.00	6.97
Thyme extract (50 mg/kg)-thyme powder (150 mg/kg)	1032.58	162.29	183.52	213.53	5.63
Thyme extract (50 mg/kg)-thyme powder (250 mg/kg)	1094.97	151.50	196.68	142.74	5.57
Thyme extract (100 mg/kg)-thyme powder (150 mg/kg)	1058.90	268.55	162.83	114.76	6.50
Thyme extract (100 mg/kg)-thyme powder (250 mg/kg)	1561.48	164.05	210.86	186.25	7.40

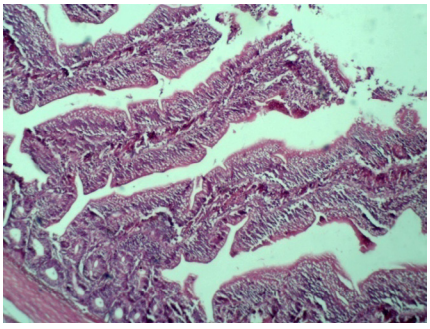


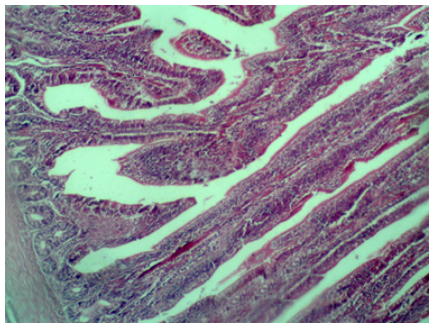
Thyme extract (0 mg/kg)-thyme powder (0 mg/kg)



Thyme extract (50 mg/kg)-thyme powder (150 mg/kg)







Thyme extract (50 mg/kg)-thyme powder (250 mg/kg) Thyme extract (100 mg/kg)-thyme powder (150 mg/kg) Thyme extract (100 mg/kg)-thyme powder (250 mg/kg)

Figure 1. Morphological image of jejunum of Ross 308 broilers in diets on day 42 with diets containing different levels of thyme extract and thyme powder from 1st-42nd days of age

wort and phenolic compounds in the leaves of Zataria multiflora (such as thymol and carvacrol) have not been shown to significantly change the titers of Newcastle and influenza antibodies (Lee et al., 2003a) which does not correspond to the results obtained from the present experiment.

Intestinal morphology

There is not significant different for villi characteristics based on the data obtained in this study that are shown in Table 11. However, numerically, the highest villi length, crypt depth, and villi width were observed relative to the crypt depth using TE (100) + TP (250). The highest villi width was observed for TE (100) + TP (150) and the highest layer thickness was observed for TE (50) + TP (150).

Garcia et al. (2007) reported that the use of herbs in the diet increased the villi height in broilers. The researchers suggested that by introducing herbs into the diet, the total population of harmful bacteria in the intestinal wall was reduced, thereby reducing the production of toxic compounds and damaging the cells lining the intestinal lining, so that the villi became longer and the crypt deepened. This reaction can cause changes in the morphology of the gut. After 42 days, Garcia et al. (2007) did not observe any significant differences in villi length and crypt depth in the jejunum between plant extracts containing cinnamaldehyde, carvacrol and capsaicin, which matched the results of the present experiment.

Aydin and Yildiz G. (2020) and also Bahrami et al., (2020) demonstrated the positive effects of thyme oil in broiler feeding on intestinal histomorphology. Khattak et al. (2014) used different levels of plant essential oils (basil, cumin, bay leaf, oregano, tea and thyme) in the diet of broilers and found that at the level of 300 g/ton, cecal villus width and surface area level also increased. The height of villi in the cecum of chickens fed diets supplemented with 100 mg/kg of plant essential oil increased compared to the control group (320 μm vs. 291 μm) ($P<0.005$). Cecal villi surface area was increased significantly ($P<0.01$) in

chickens receiving treatments of 200, 300 and 500 mg of essential oil per kg of feed compared to the control, which is consistent with the results of the present experiment.

CONCLUSION

In general, in the present study, it can be concluded that the use of thyme powder and thyme extract in the diet of Ross 308 broilers improved feed intake, daily weight gain, total conversion rate and production index. It was not very effective on blood parameters and liver enzymes but led to the improvement of the immune system and reduction of abdominal fat, and thus improved meat quality. Also, the use of high levels of TP and TE improved the morphological parameters of intestinal jejunum. Therefore, according to the results of this experiment, it is suggested that a mixture of TP and TE be used as two antioxidant, antimicrobial and inexpensive growth stimulants.

ACKNOWLEDGMENTS

This manuscript is based on a PhD thesis presented by the first author to Rasht Branch, Islamic Azad University, Rasht, Iran. Financial support by Rasht Branch, Islamic Azad University, grant number 17.16.4.18418 is gratefully acknowledged.

ETHICAL APPROVAL

The study was approved by the ethics committees of the authors' institutions.

FUNDING

Financial support by Rasht Branch, Islamic Azad University, grant number 17.16.4.18418 is gratefully acknowledged.

COMPETING OF INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Abazari F, Rezaei M, Rahimi M (2011) The effect of using different levels of thyme in low protein diet on performance and carcass characteristics of broilers. 5th Iranian Congress of Animal Science Proceedings. 39-33.

Abdulkarimi R, Aghazadeh AM, Daneshyar M (2011) Growth performance and some carcass charactristics in broiler chickens supplemented with Thymus extract (*Thymus vulgaris*) in drinking water. Journal of American Science. *7*(11): 400-405.

Ahmadian A, Seidavi AR, Phillips CJC. (2020) Growth, carcass composition, haematology and immunity of broilers supplemented with sumac berries (*Rhus coriaria* l.) and thyme (*Thymus vulgaris*). Animals. 10(3): 513, 1-14.

Aiatollahi S, Kermanshahi H, Zadeh Heravi M (2016) The effect of different levels of thyme pulp on blood parameters and serum liver enzymes of broiler blood. 7th Iranian Congress of Animal Science Proceedings. 126-135.

Alcicek A, Bozkurt M, Çabuk M (2004) The effect of a mixture of herbal essential oils, an organic acid or a probiotic on broiler performance. South African Journal of Animal Science. 34(4): 217-222.

Al-Kassie GA (2009) Influence of two plant extracts derived from thyme and cinnamon on broiler performance. Pakistan Veterinary Journal. 29(4): 169-173.

Almremdhy HAAE, Al-khafaji MA. (2020) Drinking Thyme Extract on Growth Performance and Immune Response of Broiler Chickens. Indian Veterinary Journal. 97(04): 32-35.

Aydin ÖD, Yildiz G. (2020) The effects of thyme oil and black cumin oil in broiler feeding on growth performance, intestinal histomorphology, and cecal volatile fatty acids. Turkish Journal of Veterinary and Animal Sciences, 44(1): 17-25.

Bahrami MR, Hajkhodadadi I, Ghasemi HA. (2020) Evaluation of histomorphometry of gasterointestinal tract, liver and hematology of broiler chickens treated with dietary pure and encapsulated thyme essence. Iranian Veterinary Journal. 16(1): 15-24.

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils-a review. Food Chemistry and Toxicology. 46(2): 446-475.

Behboudi H, Esmaeilipour O, Mirmahmoudi R, Mazhari M. (2016) The influence of drinking water containing lemon juice and thyme supplemented diet on performance and some blood parameters of broilers under heat stress. Iranian Journal of Applied Animal Science. 6(1), 169-174.

Beheshti Khajeh D, Nobakht A (2010) Investigation the effects of using different mixtures of peppermint (*Mentha piprita*), thyme (*Thymus vulgaris*) and saturea (*Satureia hortensis*) medicinal plants on performance, egg quality, blood and immuity parameters of laying hens. 4th Iranian Congress of Animal Science Proceedings. 790-797.

Belali M, Seidavi A, Bouyeh M. (2021) Effects of thyme powder and aqueous extract on fatty acid profile of breast meat in broiler chicks. Meso. 23(1): 38-43.

Cross DE, McDevitt RM, Hillman K Acamovic T (2007) The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. British Poultry Science. 48(4): 496-506

Demir E, Sarica Ş, Özcan MA, Sui Mez M (2003) The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. British Poultry Science. 44(S1): 44-45.

Denli M, Okan F Uluocak AN (2004) Effect of dietary supplementation of herb essential oils on the growth performance, carcass and intestinal characteristics of quail (*Coturnix coturnix* japonica). South African Journal of Animal Science. 34(3): 174-179.

Garcia V, Catala-Gregori P, Hernandez F, Megias, MD, Madrid J (2007) Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. Journal of Applied Poultry Research. 16(4): 555-562.

Gholami M, Chamani M, Seidavi A, Sadeghi AA, Aminafschar M (2020) Effects of stocking density and environmental conditions on performance, immunity, carcase characteristics, blood constitutes, and economical parameters of Cobb 500 strain broiler chickens. Italian Journal of Animal Science. 19(1): 524-535.

Ghoreyshi SM, Omri B, Chalghoumi R, Bouyeh M, Seidavi AR, Dadashbeiki M, Lucarini M, Durrazo A, van den Hoven R, Santini A (2019) Effects of dietary supplementation of l-carnitine and excess lysine-methionine on growth performance, carcass characteristics, and immunity markers of broiler chicken. Animals. 9(6): 362.

Golrokh AJ, Bouyeh M, Seidavi A, van den Hoven R, Laudadio V Tufarelli V (2016) Effect of different dietary levels of atorvastatin and L-carnitine on performance, carcass characteristics and plasma constitutes of broiler chickens. Journal of Poultry Science. 53(3): 201-207.

Hernandez F, Madrid J, Garcia V, Orengo J, Megias MD (2004) Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poultry Science. 83(2): 169-174.

Kalantar M, Saki AA, Zamani P, Aliarabi H (2011) Effect of drinking thyme essence on performance, energy and protein efficiency and economical indices of broiler chickens. Pajouhesh Sazandegi in Animal Science. 92: 59-67.

Khattak F, Ronchi A, Castelli P, Sparks N (2014) Effects of natural blend of essential oil on growth performance, blood biochemistry, cecal morphology, and carcass quality of broiler chickens. Poult Sci. 93(1): 132-137.

Lee KW, Everts H, Kapperst HJ, Yeom KH, Beynen AC (2003a) Dietary carvacrol lowers body weight gain but improves feed conversion in female broiler chickens. Journal of Applied Poultry Research. 12(4): 394-399.

Lee KW, Everts H, Kappert HJ, Frehner M, Losa, R, Beynen AC (2003b) Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. British Poultry Science. 44(3): 450-457.

Madrid J, Hernandez F, Garcia V, Orengo J, Megias MD, Sevilla V (2003) Effect of plant extracts on ileal apparent digestibility and carcass yield in broilers at level of farm. In 14th European Symposium of Poultry Nutrition. Lillehammer, Norway. 187-188.

Manzanilla EG, Baucells F, Kamel C, Morales J, Perez JF, Gasa J (2001) Effects of plant extracts on the performance and lower gut microflora of early weaned piglets. Journal of Animal Science Suppl, 1 (Abstract): 473.

Ocak N, Erener G, Burak Ak, F, Sungu M, Altop A, Ozmen A (2008) Performance of broilers fed diets supplemented with dry peppermint (*Mentha piperita* L.) or thyme (*Thymus vulgaris* L.) leaves as growth promoter source. Czech Journal of Animal Science. 53(4): 169-175.

Pourhossein Z, Qotbi AAA, Seidavi A, Laudadio V, Centoducati G, Tufarelli V (2015) Effect of different levels of dietary sweet orange (*Citrus sinensis*) peel extract on humoral immune system responses in broiler chickens. Animal Science Journal. 86(1): 105-110.

Pournazari M, Qotbi A, Seidavi A, Corazzin M (2017) Prebiotics, probiotics and thyme (*Thymus vulgaris*) for broilers: Performance, carcass traits and blood variables. Revista Colombiana de Ciencias Pecuarias. 30(1): 3-10.

Rafat Khafar K, Mojtahedin A, Rastegar N, Kalvani Neytali M, Olfati A. (2019) Dietary inclusion of thyme essential oil alleviative effects of heat stress on growth performance and immune system of broiler chicks. Iranian Journal of Applied Animal Science. 9(3), 509-517.

Rafiee A, Rahimian Y, Zamani F, Asgarian F (2013) Effect of use ginger (*Zingiber officinale*) and thymus (*Thymus vulgaris*) extract on performance and some hematological parameters on broiler chicks. Science of Agriculture. 4(1): 20-25.

Rahimi S, Teymouri ZZ, Karimi TM, Omidbaigi R, Rokni H (2011) Effect of the three herbal extracts on growth performance, immune system, blood factors and intestinal selected bacterial population in broiler chickens. ournal of Agricultural Science and Technology. 13: 527-539

Sarica S, Ciftci A, Demir E, Kilinc K, Yildirim Y (2005) Use of an antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat based broiler diets. South African Journal of Animal Science. 35(1): 61-72.

Sharififar F, Moshafi MH, Mansouri SH, Khodashenas M, Khoshnoodi M (2007) *In vitro* evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic Zataria multiflora Boiss. Food Controls. 18(7): 800-805.

Souri H, Khatibjoo A, Taherpoor K, Hassan Abadi A, Fattahnia F, Askari M. (2015) Effect of *Thymus vulgaris* and *Satureja khuzestanica* ethanolic extracts on broiler chickens' performance and immune response. Iranian Journal of Applied Animal Science. 5(2), 437-446.

Tymorizade Z, Rahimi S, Thorshizi MK (2010) The comparison of Virginymicin antibiotic and three commercial plants extract on blood parameters in Ross broiler chickens. Abstract. 3rd Iranian Congress of Animal Science Proceedings. 177-182.

Williams P (2001) The use of essential oils and their compounds in poultry nutrition. World Poult. 17: 14-15.

Zahirian M, Seidavi A, Solka M, Nosrati M, Corazzin M (2019) Dietary supplementation of Aspergillus oryzae meal and its effect on performance, carcass characteristics, blood variables, and immunity of broiler chickens. Tropical Animal Health and Production. 51(8): 2263-2268.