Effects of short-term and combined use of thyme powder and aqueous extract on growth performance, carcass and organ characteristics, blood constituents, enzymes, immunity, intestinal morphology and fatty acid profile of breast meat in broilers

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SUMMARY

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, intestinal morphology and fatty acid profile of breast meat in broilers. The experiment was based on a completely randomized design with 5 treatments, 4 replications and 10 Ross 308 male broilers in each replication for 42 days. Experimental treatments included aqueous extract of thyme (50 and 100 ppm) and thyme powder (100 and 150 mg/kg/feed) which were used in combination with the basal diet (control). The effect of treatments was analyzed by analysis of variance (SAS) and the means were compared at 5% probability level with Duncan's multiple range test. The results showed that, in the final period of experiment, different levels of thyme aqueous extract and thyme powder had a significant difference on daily weight gain, feed intake and conversion ratio improvement ($P<0.05$) so that the highest means were related to treatment TE (50 ppm) - TP-(150 mg/kg). The effect of different levels of thyme aqueous extract and thyme powder had a significant difference on European index, economic value and total weight ($P<0.05$), which had the highest mean of TE (50 ppm) -TP (150 mg/kg). Different levels of thyme aqueous extract and thyme powder had significant differences on the relative weight of thymus, bursa of Fabricius, live weight, ventricular fat and pancreas ($P<0.05$). The effect of different levels of thyme aqueous extract and thyme powder on the performance of the immune system of broilers was not significant ($P> 0.05$). The effect of different levels of thyme aqueous extract and thyme powder on the performance of the immune system of broilers was not significant ($P>0.05$). The highest percentage of unsaturated fatty acids was related to high levels of thyme powder and extract, meaning that the highest mean was related to TE (100 ppm) -TP (250 mg/kg) and the lowest mean was related to TE (0 ppm). TP (0 mg/kg). Based on the results of the present study, the use of TE 50 ppm, TP 150 mg/kg level is recommended to supplement the diet of Ross 308 broilers.

KEY WORDS

Medicinal plants, thyme, chick, growth, breast meat, antibody.

INTRODUCTION

The use of drugs and chemicals as growth stimulants in livestock and poultry diets has been officially banned by the European Union since 2006. In recent years, the use of natural and organic substances as alternatives has increased sharply following the imposition of legal restrictions on the use of drugs and chemicals. Studies have shown that herbs and their products, known as phytobiotics, are good alternatives to growth-promoting antibiotics in the diet or drinking water of broilers (Griggs *et al.*, 2005; Grashorn, 2010; Hashemi *et al.* 2010; Windisch *et al.* 2008). The use of antibiotics has been banned due to drug resistance in humans, drug retention in the carcass, and disturbance of the normal intestinal microflora unless necessary for treatment. In many parts of the world there are recommendations to discourage the use of growth-promoting antibiotics, so to compensate for this reduction in growth, it is necessary to find suitable alternatives. In recent years, the use of aromatic plants and their extracts as potential growth stimulants has attracted much attention (Ghazanfari *et al.* 2015). Medicinal plants due to factors such as high economic value and low cost of production, little effect on the environment, few side effects compared to chemical drugs and antibiotics and reduced relative resistance to pathogens. Some of the beneficial properties of medicinal plants are related to the presence of secondary metabolites such as phenolic compounds, essential oils and saponins. (Tipu *et al.* 2006).

Thyme (*Thymus vulgaris* L) is a herbaceous, aromatic plant belonging to the mint family. Thymol and carvacrol are important active ingredients, but other substances such as paracetamol, linalool and cineole, flavonoids, terpenes, spicy compounds and a number of other active ingredients are found (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) reported that thyme oil had a positive effect on the performance

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of broilers.

Al-Kassie, (2009) investigated the effect of thyme and cinnamon extracts at 100 and 200 mg in broilers and reported that growth performance traits (daily weight gain, feed intake and feed conversion ratio) were significantly higher for birds that received these two extracts than the control group, and the higher levels of extracts showed higher results than lower levels. Herandez *et al.* (2004) showed that feeding plant extracts such as thyme cause faster growth, improve intestinal digestion, starch digestibility, dry matter utilization of diets and carcass traits in broilers.

Thyme extract has high antioxidant properties that, in addition to reducing blood lipids, can play a role in inhibiting LDL oxidation. Carvacrol reduces plasma triglyceride concentrations. The use of carvacrol has been reported to stimulate the growth and proliferation of lactobacilli, and lactobacilli play an important role in improving blood parameters and lowering serum lipids (Esteve *et al.* 2000).

The most important phenolic compounds in thyme include thymol and carvacrol. This plant has antibacterial, antifungal and anti-coccidiotic properties that are attributed to thymol and carvacrol Mikaili *et al.* (2010).

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. 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 food intake by increasing absorption through intestinal villi (Denli *et al.* 2004).

There are limited results regarding the simultaneous evaluation of the effects of thyme powder and its extract in the diet of broilers in the short term and to investigate the possibility of reducing the cost of diet, treatments were used in the short term. The present experiment was performed to evaluate the effects of thyme powder and its extract on growth performance, carcass and gastrointestinal characteristics, blood parameters, immune system, intestinal morphology and profile of breast fatty acids in broiler chickens.

MATERIALS AND METHODS

This study was conducted in one of the broiler farms located in Masal, Iran. The average weight of 45 ± 2 g with control treatment was used in a total of 5 treatments and four replications and 10 chickens per replication for 42 days. Two levels of thyme powder (150 and 250 mg/kg) and two levels of aqueous thy-

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

me extract (at levels of 50 and 100 ppm) in the diet in the first 24 days of feeding were applied as follows. The treatments were:

Treatment 1: Aqueous extract of thyme (0 ppm) + Thyme powder (0 mg/kg) Treatment 2: Aqueous extract of thyme (50 ppm) + Thyme powder (150 mg/kg) Treatment 3: Thyme aqueous extract (50 ppm) + Thyme powder (250 mg/kg) Treatment 4: Thyme aqueous extract (100 ppm) + Thyme powder (150 mg/kg) Treatment 5: Aqueous extract of thyme (100 ppm) + thyme powder (250 mg/kg) which was used in combination with the basic diet.

Aqueous extract of thyme and thyme powder made by Zarghani Pharmaceutical Company (Sabzevar, Iran) were purchased and used based on the desired concentrations. Diets were adjusted according to the poultry nutritional requirements table containing the minimum nutrients recommended in the Ross 308 strain feeding guide Manual, (2012) (Table 1). Chickens were reared in 1 × 1 m cages on a cellulose roll bed for 42 days. Each cage had a cylindrical feeder and a manual feeder. The temperature of the breeding hall decreased to 33 degrees Celsius in the first days and then gradually to 23 degrees Celsius 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 gain of chickens per pen at periods of 1 to 10, 11 to 24 and 25 to 42 days was calculated by a digital scale 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. Ghoreyshi *et al.* (2019) used the following formula to measure 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, by a digital scale with an accuracy of ± 0.1 g and stuffed carcass weight, full carcass weight, empty carcass weight, breast weight, weight thigh, wing weight as well as the weight of internal organs (pancreas, heart, gills, spleen, bursa of Fabricius, liver, ventricular fat, weight of duodenum, geogenome and ileum) were measured (Zahirian *et al.* 2019).

Blood serum parameters and digestive enzymes

At the end of the experiment (42 days old), 2 birds weighing close to the average were randomly selected and blood samples were taken from the wing vein of 5 ml. The samples were centrifuged at room temperature at 5000 rpm for 3 minutes (5702, Eppendorf, Germany) and serum was separated and transferred to microtubes and transferred to the laboratory. The serum was stored at minus 20 ° C until blood metabolites were measured. Serum was thawed at room temperature followed by 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 erythrocytes (SRBC according to the theory of Lerner *et al.* (1971)). To prepare an SRBC injection suspension, blood samples were taken from 3 sheep and poured into jars containing EDTA. Rinses were washed three times in PBS saline phosphate buffer, and at the end of a suspension, 2% SRBC was prepared in PBS. All the above steps were performed under sterile conditions. Then it was injected into the vein of the above solution 7 and 14 days after the first and second injections and blood samples were taken on days 35 and 42 (Gore and Qureshi, 1997). SRBC was measured by hemagglutination method. To measure the antibody titer, V-shaped pellets for microhemagglutination were prepared, which have 96 wells in 12 columns (1 to 12) and 8 rows (A to H). Van derzipp method was used to measure total antibody. According to this method for measuring Total anti-SRBC, ul 50 of the serum sample was mixed with ul 50 saline phosphate buffer (PBS) inside the microtiter plate and in the next step, 50% of 2% SRBC suspension solution was added to each well and then placed at room temperature for 4 to 5 hours. Titers were expressed based on log2. 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). NDV) and influenza Blood samples were taken from 2 birds per pen on 28 and 42 days and then 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 nests, then 25 microliters of bird serum was diluted in the first nest and diluted to the last nest. The mechanical shaker was placed and the microplate was placed at 25 ° C for 30 minutes, then 25 µl of 1% red blood cells was added to all the lumps 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. Pastel was used for HI test. The titles were diluted based on log2. The 1% 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

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-24th 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/da)	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 ppm)-thyme powder (0 mg/kg)	18.10	9.65^{b}	1.88	48.40^{c}	32.71^{b}	1.48	144.83	68.57	2.11	82.52^{b}	42.59^{b}	1.94^{a}
Thyme extract (50 ppm)-thyme powder (150 mg/kg)	22.45	13.15^{a}	1.70	64.10^{ab}	49.29^{a}	1.30	155.75	79.19	1.97	93.46^{a}	53.50^{a}	1.75^{b}
Thyme extract (50 ppm)-thyme powder (250 mg/kg)	20.92	11.3^{ab}	1.85	60.89^{ab}	46.20^{a}	1.35	156.09	81.12	1.94	92.17^{a}	52.86^{a}	1.74^{b}
Thyme extract (100 ppm)-thyme powder (150 mg/kg)	18.80	11.6^{ab}	1.61	57.85^{b}	45.06^{a}	1.29	153.02	78.95	1.94	89.34^{ab}	51.61^{a}	1.73^{b}
Thyme extract (100 ppm)-thyme powder (250 mg/kg)	18.40	12.65^{a}	1.48	65.39^{a}	45.43^{a}	1.46	155.31	80.11	1.95	92.74^{a}	52.49^{a}	1.77^{b}
P-value	0.17	0.01	0.09	0.0004	0.003	0.48	0.70	0.38	0.21	0.03	0.003	0.0002
SEM	1.37	0.65	0.10	2.15	2.46	0.09	6.33	4.81	0.06	2.4	1.75	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

were collected from each pen and their blood was transferred to tubes containing anticoagulant. Blood cells were determined by Cell staining, differentiation and eye counting were performed under a light microscope (Golrokh *et al.* 2015).

Intestinal morphology

At 42 days of age, immediately after slaughter, tissue sections of the 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 hematoxylin and eosin (HE) method. Xylol (10 minutes each), 96% alcohol (5 minutes), 100% alcohol (5 minutes) and hematoxylin (10 minutes), 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. (Ganjeh *et al.* 2016).

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

Profile of fatty acids of breast meat

Determination of breast fatty acid profile was performed by extracting 10 g of breast fat from 1 chicken in each treatment. At first, the fat samples were mixed well with 100 ml of methanol: chloroform (2: 1) solution for about 3 to 4 hours. The purified samples were then mixed with 25 ml of sodium chloride saturated solution in a decanter funnel. In the next step, the chloroform phase containing the fat was removed by filter paper impregnated with potassium anhydrous sulfate. The filtered sample was dried under vacuum by a rotating operator to leave only fat. After this step, 10 mg of extracted fat was stirred well with 2 ml of potassium hydroxide, 2 ml of normal methanol and 7 ml of -n hexane, then the resulting samples were centrifuged for 10 minutes. In the next step, the sample remained stationary for 5 minutes to separate the top phase. Then about one microliter of the supernatant was injected into the gas chromatography apparatus to evaluate the profile of fatty acids and the amount of the above fatty acids was expressed as a percentage of total fatty acids (Tavakoli *et al.* 2020).

Statistical analysis

At the end of the experiment, the obtained data were statistically analyzed using analysis of variance by SAS statistical software. The mean of treatments was compared at 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 given in Tables 2 and 3. In the period of 1 to 10 days, different levels of thyme aqueous extract and thyme powder did not differ significantly in feed intake and conversion ratio improvement (P> 0.05), but there was a significant difference in daily weight gain (P<0.05, which was the highest mean of treatment). TE (50

Table 3 - Economic performance mean (±SEM) of Ross 308 broilers at 42 days of age fed diets containing the different levels of thyme extract and thyme powder from 1st-24th days of age.

	Weight of 1 chick at 42th days of age (g/chick)	Feed cost per kg live weight (Rial/kg)	European production index
Thyme extract (0 ppm)-thyme powder (0 mg/kg)	1828.75^{b}	52978.30^{a}	224.90^{b}
Thyme extract (50 ppm)-thyme powder (150 mg/kg)	2287.00^{a}	48429.60^{b}	312.40^{a}
Thyme extract (50 ppm)-thyme powder (250 mg/kg)	2260.00^{a}	48174.80^{b}	308.71^{a}
Thyme extract (100 ppm)-thyme powder (150 mg/kg)	2207.90^{a}	47894.10^{b}	303.62^{a}
Thyme extract (100 ppm)-thyme powder (250 mg/kg)	2244.50^{a}	49107.40^{b}	303.15^{a}
P-value	0.003	0.0007	0.001
SEM	73.42	702.59	13.04

* 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

ppm) - TP (150 mg/kg) and the lowest mean was related to TE (0 ppm) -TP (0 mg/kg). In the period of 11 to 24 days, different levels of thyme aqueous extract and thyme powder did not differ significantly in 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 25 to 42 days. There was no significant difference between thyme aqueous extract and thyme powder on daily weight gain, feed intake and conversion ratio (P> 0.05). In the period of 1 to 42 days, different levels of thyme aqueous extract and thyme powder had a significant difference on daily weight gain, feed intake and conversion ratio improvement (P<0.05), which was the best mean for TE (50 ppm) -TP (150 mg/kg) treatment and the weakest mean was related to TE (0 ppm) -TP (0 mg/kg) treatment i.e. the control group. The effect of different levels of thyme aqueous extract and thyme powder was significantly different on European index, economic value and total weight of the period (P<0.05). Disadvantages include the presence of harmful microbes in the gastrointestinal tract, increased degradation of proteins and amino acids in the gastrointestinal tract due to the deamination activity of microbes, and increased rate of their decomposition due to the secretion of substances such as microbial urease enzyme. Medicinal plants reduce the microbial population of the gastrointestinal tract, thus slowing down the breakdown of proteins and amino acids in the digestive tract and absorbing larger amounts of them, thereby improving feed conversion ratio (Lee *et al.* 2003b). Antioxidants in medicinal plants, by protecting the intestinal villi, improve the absorption of nutrients and thereby improve the performance of the bird, which is consistent with the results of the present experiment (Manzanillo *et al.* 2001).

In an experiment, chickens that received alcoholic extract of thyme 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. Digestion of thyme extract with its low pH. Manzanillo *et al.* (2001) also reported that beneficial antioxidant compounds of medicinal plants protect intestinal villi by improving nutrient uptake, thereby improving bird performance. Kalantar *et al.* (2011) also used thyme essential oil in drinking water and at the end of the experiment, they reported the best conversion ratio of 0.2% thyme, 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, which is consistent with the results of the present experiment.

Hamdieh *et al.* (2013) and Okac *et al.* (2008) did not observe a significant difference in feed intake in any of the experimental courses by adding dried thyme powder and thyme essential oil to the diet of broilers.

Characteristics of carcasses and some organs

The effect of experimental treatments on carcass characteristics is shown in Tables 4 and 5. The results showed that the use of different levels of aqueous extract of thyme and thyme powder had a significant difference on the relative weight of thymus, bursa of Fabricius, live weight, ventricular fat and pancreas (P<0.05). It has been reported that the consumption of medicinal plants stimulates the growth of immune organs of broilers and causes a significant increase in their weight (Takahashi *et al.* 2000). The presence of bioactive compounds in thyme probably stimulates cell proliferation in these organs. The bursa of Fabricius, thymus, and spleen are among the organs of the immune system, and improving the weight of each can improve the condition of the bird's immune system. Perhaps the higher relative weight in the bursa of Fabricius and

Table 4 - Mean (±SEM) of economically relevant carcass characteristics of Ross 308 broilers at 42 days of age fed diets containing the different levels of thyme extract and thyme powder from 1st-24th 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 ppm)-thyme powder (0 mg/kg)	2267.50^{b}	2031.80^{b}	1869.30^{b}	1596.50^{b}	78.53^{b}	0.44	26.93	21.99	8.27	1.65^{a}	0.31^{a}
Thyme extract (50 ppm)-thyme powder (150 mg/kg)	2640.00^{a}	2436.30^{a}	2320.00^{a}	2032.50^{a}	83.42^{a}	1.06	29.30	22.09	6.69	0.94^{ab}	0.28^{ab}
Thyme extract (50 ppm)-thyme powder (250 mg/kg)	2760.00^{a}	2545.00^{a}	2296.30^{a}	2112.50^{a}	83.03^{a}	0.54	30.45	21.80	6.26	0.81^{b}	0.22^{bc}
Thyme extract (100 ppm)-thyme powder (150 mg/kg)	2685.00^{a}	2502.50^{a}	2345.00^{a}	2107.50^{a}	84.06^{a}	0.89	31.08	21.11	6.02	0.55^{b}	0.18^{c}
Thyme extract (100 ppm)-thyme powder (250 mg/kg)	2845.00^{a}	2700.00^{a}	2515.00^{a}	2211.80^{a}	82.08^{a}	0.75	29.56	20.56	6.10	0.53^{b}	0.21^{bc}
P-value	0.02	0.01	0.006	0.004	0.02	0.19	0.40	0.75	0.05	0.03	0.03
SEM	110.68	114.71	102.33	98.10	1.10	0.19	1.53	0.95	0.53	0.24	0.03

* 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 42 days of age fed diets containing the different levels of thyme extract and thyme powder from 1st-24th days of age.

	Relative weight of gizzard (ventriculu) (%)	Relative weight of heart (%)	Relative weight of liver (%)	Relative weight of proventriculus (%)
Thyme extract (0 ppm)-thyme powder (0 mg/kg)	2.18	0.75	2.56	0.48
Thyme extract (50 ppm)-thyme powder (150 mg/kg)	2.66	0.63	2.51	0.44
Thyme extract (50 ppm)-thyme powder (250 mg/kg)	2.54	0.61	2.38	0.43
Thyme extract (100 ppm)-thyme powder (150 mg/kg)	2.36	0.51	2.46	0.43
Thyme extract (100 ppm)-thyme powder (250 mg/kg)	2.57	0.56	2.68	0.45
P-value	0.17	0.23	0.95	0.83
SEM	0.14	0.07	0.27	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

thymus indicates the effect of thyme extract on the bird's immune status.

Hamdieh *et al.* (2013), Abazari *et al.* (2011), Rahimi *et al.* (2011), using a mixture of plant essential oils 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 thyme alcoholic extract supplement on growth performance and some carcass traits was investigated and it was reported that the relative wing weight of chickens that received the 4% level was significantly higher than the chickens that did not receive. Thyme extract in drinking water significantly increased the relative weight of breast and wings. Abdulkarimi *et al.* (2011) concluded that consumption of alcoholic thyme extract in drinking water improved the performance and relative weight of broiler chickens which does not agree with the results obtained in the present experiment.

Intestinal parts

The effect of experimental treatments on different parts of the intestine is shown in Table 6. The use of different levels of aque-

Table 6 - Mean (±SEM) of intestinal segments of Ross 308 broilers at 42 days of age fed diets containing the different levels of thyme extract and thyme powder from 1st-24th 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 ppm)-thyme powder (0 mg/kg)	0.24^{a}	0.72	1.29^{a}	0.54	0.38^{a}	0.19	0.19
Thyme extract (50 ppm)-thyme powder (150 mg/kg)	0.23^{a}	0.67	0.99^{bc}	0.50	0.24^{c}	0.16	0.16
Thyme extract (50 ppm)-thyme powder (250 mg/kg)	0.17^{b}	0.75	1.08^{b}	0.54	0.33^{ab}	0.17	0.18
Thyme extract (100 ppm)-thyme powder (150 mg/kg)	0.19^{ab}	0.59	1.06^{b}	0.50	0.25^{bc}	0.17	0.16
Thyme extract (100 ppm)-thyme powder (250 mg/kg)	0.18^{b}	0.82	0.87^{c}	0.54	0.29^{bc}	0.16	0.15
P-value	0.03	0.36	0.001	0.92	0.01	0.39	0.35
SEM	0.02	0.08	0.06	0.05	0.03	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 constituents mean (±SEM) of Ross 308 broilers at 42 days of age fed diets containing the different levels of thyme extract and thyme powder from 1st-24th 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 ppm)-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 ppm)-thyme powder (150 mg/kg)	158.75	76.75	15.35	84.00	45.50	0.54	191.75	3.70	1.85	1.85
Thyme extract (50 ppm)-thyme powder (250 mg/kg)	146.25	61.50	12.30	79.25	38.00	0.48	145.25	3.60	2.22	1.37
Thyme extract (100 ppm)-thyme powder (150 mg/kg)	133.50	78.50	15.70	74.50	33.50	0.45	191.00	3.75	1.85	1.90
Thyme extract (100 ppm)-thyme powder (250 mg/kg)	177.75	64.50	12.90	88.50	53.75	0.60	170.00	3.72	1.77	1.95
P-value	0.05	0.93	0.93	0.10	0.06	0.12	0.15	0.99	0.50	0.09
SEM	10.96	17.13	3.42	4.32	4.85	0.04	15.42	0.31	0.32	0.19

* 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 8 - Liver enzymes mean (±SEM) of Ross 308 broilers at 42 days of age fed diets containing the different levels of thyme extract and thyme powder from 1st-24th days of age.

	Alkaline phosphatase (U/L)	Alanine transaminase (IU/L)	Lactate dehydrogenase (IU/L)	Creatine kinase (IU/L)
Thyme extract (0 ppm)-thyme powder (0 mg/kg)	5048.30	239.25^{b}	4280.00^{b}	10051.00^{b}
Thyme extract (50 ppm)-thyme powder (150 mg/kg)	4269.00	351.75^{b}	4588.50^{b}	18470.00^{b}
Thyme extract (50 ppm)-thyme powder (250 mg/kg)	2945.30	514.50^{a}	7749.00^{a}	47025.00^{a}
Thyme extract (100 ppm)-thyme powder (150 mg/kg)	4919.50	320.25^{b}	5318.30^{b}	15005.00^{b}
Thyme extract (100 ppm)-thyme powder (250 mg/kg)	5538.50	278.25^{b}	4158.00^{b}	9802.00^{b}
P-value	0.09	0.002	0.01	0.0005
SEM	642.97	40.02	687.87	5064.26

* 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-24th 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 ppm)-thyme powder (0 mg/kg)	5250.00	14.25	80.00	5.50	4.00	6.00	2.50	4.50	5.50	6.75
Thyme extract (50 ppm)-thyme powder (150 mg/kg)	1925.00	21.50	72.50	6.00	3.00	5.75	2.75	5.00	5.00	7.00
Thyme extract (50 ppm)-thyme powder (250 mg/kg)	2575.00	36.25	56.75	7.00	2.50	5.50	2.00	4.50	4.50	6.50
Thyme extract (100 ppm)-thyme powder (150 mg/kg)	2200.00	31.75	63.50	4.75	3.00	5.75	2.50	5.00	5.00	7.00
Thyme extract (100 ppm)-thyme powder (250 mg/kg)	3600.00	20.75	74.75	4.50	1.50	5.50	2.50	5.25	5.25	7.25
P-value	0.34	0.15	0.19	0.85	0.21	0.98	0.55	0.72	0.64	0.81
SEM	1220.55	6.40	7.00	1.74	0.71	0.62	0.31	0.46	0.46	0.46

* 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 10 - Immunity related organ mean (±SEM) of Ross 308 broilers at 42 days of age fed diets containing the different levels of thyme extract and thyme powder from 1st-24th days of age.

	Relative weight of thymus (%)	Relative weight of spleen (%)	Relative weight of bursa of Fabricius (%)
Thyme extract (0 ppm)-thyme powder (0 mg/kg)	0.48[a]	0.13	0.19[a]
Thyme extract (50 ppm)-thyme powder (150 mg/kg)	0.35[b]	0.11	0.11[bc]
Thyme extract (50 ppm)-thyme powder (250 mg/kg)	0.33[b]	0.10	0.09[c]
Thyme extract (100 ppm)-thyme powder (150 mg/kg)	0.33[b]	0.10	0.15[ab]
Thyme extract (100 ppm)-thyme powder (250 mg/kg)	0.32[b]	0.11	0.16[ab]
P-value	<0.0001	0.82	0.005
SEM	0.01	0.02	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

ous extract of thyme and thyme powder had a significant difference on the weight ratio of rectum, jejunum and colon (P<0.05), which had the highest mean level TE (0 ppm) - TP (0 mg/kg). The use of a mixture of thyme and peppermint increased the relative weight of the ileum numerically compared to the control (Denli *et al.* (2004), which did not match the results of the present experiment.

Blood parameters and liver enzymes

Data on the effect of thyme aqueous extract and thyme powder on blood parameters are given in Table 7. The results showed that the use of different levels of thyme aqueous extract and thyme powder on the blood parameters of broilers was not significant (P> 0.05). Tymorizade *et al.* (2010) reported a significant decrease in blood cholesterol and triglyceride levels in experiments on broilers 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 thyme aqueous extract and thyme powder on liver enzymes is shown in Table 8. The results showed that the effect of different levels of thyme aqueous extract and thyme powder had a significant difference on alanine transaminase, lactate dehydrogenase and creatine kinase of broilers in the whole period (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).

Immune System

The effect of different levels of aqueous extract and thyme powder on the function of the humoral immune system in response to SRBC antigen injection and antibody titers against Newcastle virus and influenza are shown in Tables 9 and 10. The results showed that the use of different levels of thyme aqueous extract and thyme powder on the performance of the immune system of broilers was not significant (P> 0.05). Beheshti *et al.* (2010) reported the use of 2% of a mixture of thyme, mint and savory in the diets of laying hens improved the performance of blood parameters and safety, which does not match the results of the present experiment.

Silymarin Phytosomes in the seed plant *Silybum marianum* or phenolic compounds in the leaves of thyme (such as thymol and carvacrol) have not been able to significantly change the titers of Newcastle disease and influenza, Gambro and bronchitis from the present experiment (Lee *et al.* 2003a).

Table 11 - Morphological indices of jejunum Ross 308 broilers in 42-day diets containing different levels of thyme extract and thyme powder from 1st-24th days of age.

	The length of the villi	The width of the vill	Crypt depth	Layer thickness	The length of the villi/crypt depth
Thyme extract (0 ppm)-thyme powder (0 mg/kg)	995	151.57	142.57	159	6.97
Thyme extract (50 ppm)-thyme powder (150 mg/kg)	1049.12	203.24	164.16	173.57	6.39
Thyme extract (50 ppm)-thyme powder (250 mg/kg)	1095.23	104.34	182.89	149.97	5.99
Thyme extract (100 ppm)-thyme powder (150 mg/kg)	1090.43	189.50	176.88	121.49	6.16
Thyme extract (100 ppm)-thyme powder (250 mg/kg)	1350.9	163.75	186.83	203.69	7.23

Table 12 - Profile of breast fatty acids Ross 308 broilers in 42-day diets containing different levels of thyme extract and thyme powder from 1st-24th days of age.

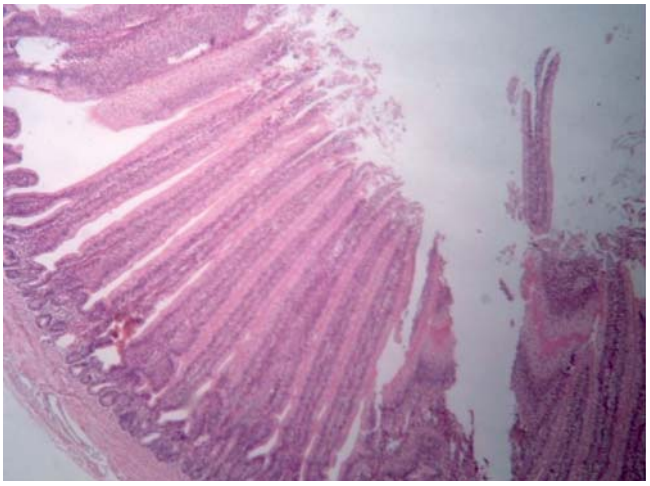
	Myristic Acid Methyl Ester C14:0 (%)	Palmitic Acid Methyl Ester C16:0 (%)	Palmitoleic Acid Methyl Ester C16:1c (%)	Stearic Acid Methyl Ester C18:0 (%)	Oleic Acid Methyl Ester C18:1n9c (%)	Linoleic Acid Methyl Ester C18:2n6c (%)	Linolenic Acid Methyl Ester C18:3n3 (%)	cis-11,14-Eicosadienoic Acid Methyl Ester C20:2c (%)	cis-8,11,14-Eicosatrienoic Acid Methyl Ester C20:3n6c (%)	cis-11,14,17-Eicosatrienoic Acid Methyl Ester C20:3 (%)
Thyme extract (0 ppm)-thyme powder (0 mg/kg)	2.02	37.48	3.14	11.40	22.57	17.65	0.50	0.38	0.67	4.18
Thyme extract (50 ppm)-thyme powder (150 mg/kg)	1.03	36.24	3.43	11.38	22.52	19.65	0.87	0.64	0.84	3.40
Thyme extract (50 ppm)-thyme powder (250 mg/kg)	2.93	35.28	2.10	12.63	22.30	19.54	0.74	0.65	0.66	3.16
Thyme extract (100 ppm)-thyme powder (150 mg/kg)	1.74	33.71	2.53	11.91	24.23	20.71	0.90	0.44	0.61	3.22
Thyme extract (100 ppm)-thyme powder (250 mg/kg)	1.08	33.58	2.82	10.70	25.20	20.60	0.90	0.60	0.76	3.76

Intestinal morphology

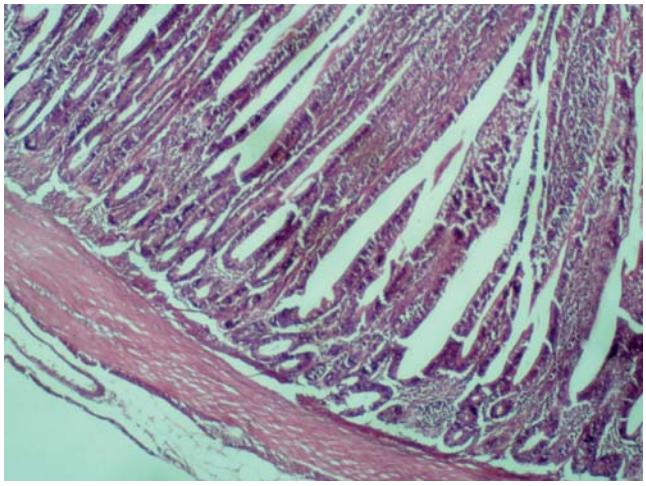
Based on the data obtained in this study, which is shown in Table 11, the highest villi length, crypt depth, layer thickness and villi length were observed relative to the crypt depth using TE (100 ppm) -TP (250 mg/kg). The highest villi width was observed in TE (50 ppm) -TP (150 mg/kg).

Garcia *et al.* (2007) reported that the use of herbs in the diet increased 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 of the intestinal lining, so that the villi became longer and the crypt deepened. This reaction can cause changes in the morphology of the gut.

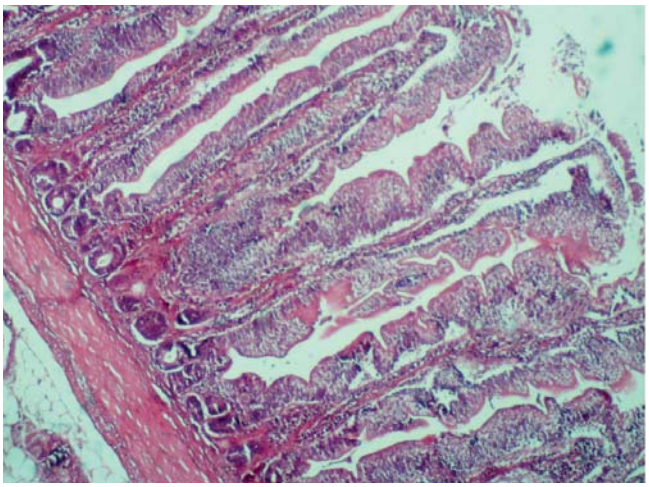
Garcı a *et al.* (2007) at 42 days of age did not observe any significant differences between plant extract treatment contain-



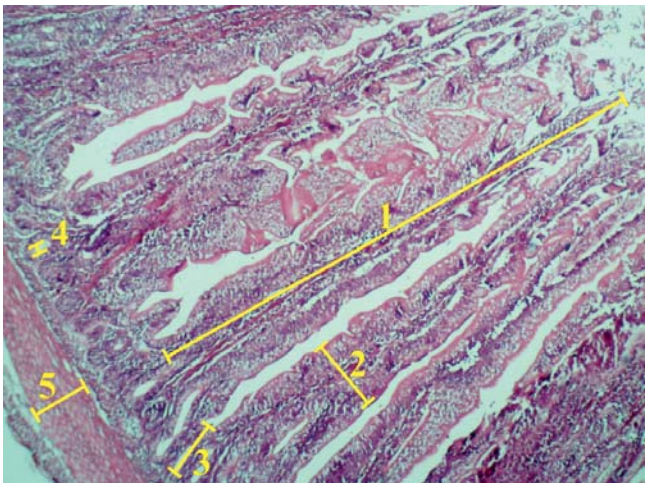
Thyme extract (50 ppm)-thyme powder (150 mg/kg)



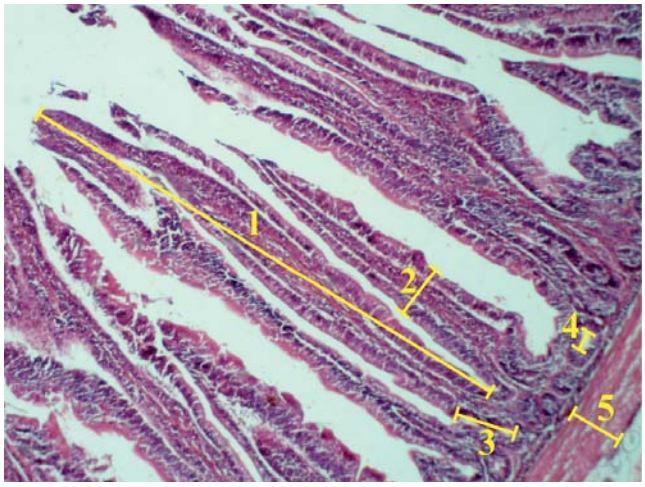
Thyme extract (50 ppm)-thyme powder (150 mg/kg)



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



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



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

Figure 1 - Morphological image of jejunum Ross 308 broilers in diets on day 42 with diets containing different levels of thyme extract and thyme powder from 1st-24th days of age.

ing cinnamaldehyde, carvacrol and capsaicin and control treatment with villi length and crypt depth in the jejunum, which matched the results of the present experiment.
Khattak *et al.* (2014) reported that they 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 the width of cecal villi and area level also increased. The height of villi in cecum of chickens fed a diet supplemented with 100 μm of plant essential oil increased compared to the control group (291 μm vs. 320 μm). ($P<0.05$). Cecum had a significant increase ($P<0.01$) 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.

Profile of fatty acids of breast meat

The percentage of fatty acids in breast muscle tissue is shown in Table 12. The results showed that the lowest percentage of three saturated fatty acids, myristic acid palmitic acid and stearic acid and also the highest level of unsaturated fatty acids was related to the highest treatment of thyme extract, except palmitoleic acid, cis-8-11-14-eicosatrienoic acid.
Cis-11,14-eicosadienoicacid that the highest percentage was observed in low levels of this extract. The results of using thyme powder showed that the highest saturated fatty acid, myristic acid, and stearic acid belonged to its highest treatment, except palmitic acid, which belonged to the lowest treatment.
The results of interaction between powder and extract showed that the lowest saturated fatty acids myristic acid and stearic acid were related to TE (50 ppm) - TP (150 mg/kg) level. Lee *et al.* (2003) also found that the amount of linoleic acid in adipose tissue increased with thyme supplementation in the diet, which is consistent with the results of the present experiment.

CONCLUSIONS

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 broiler chickens in the short term improved feed consumption, daily weight gain, total period conversion ratio and production index. Although it did not have much effect on blood parameters, it had a positive effect on liver enzymes and also led to an improvement in the immune system and a reduction in ventricular fat, thus improving the quality of meat. The use of high levels of thyme powder and thyme extract improved the morphological evaluation parameters of intestinal jejunum. The use of high levels of thyme powder and thyme extract increased saturated fatty acids and decreased some saturated fatty acids, which have health benefits. Therefore, according to the results of this experiment, it is recommended that thyme powder and thyme extract 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.

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