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Thyme Plant Powder (*Thymus vulgaris*) Improves the Production Performance of Laying Hens by Affecting Ovarian Follicles

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ABSTRACT

The present experiment investigated the effects of different levels of thyme powder on performance, blood biochemical parameters, reproductive system morphometry, and egg quality traits at different storage temperatures in old laying hens. For this purpose, in a completely randomized design, 144 laying hens (Hy-line-W36) aged 65 weeks were assigned to 3 treatments, 6 replications, and 8 birds each. Experimental diets were corn-soy based, including 0%, 0.25%, and 0.5% thyme powder (TP). At the end of the experimental period (8th week of the experiment), 6 eggs were selected from each replicate and kept at temperatures of 4 and 25 °C for 30 days to determine the internal quality. The results showed that the egg weight of birds fed with diets containing 0.25% and 0.5% TP was significantly higher than control ones (p<0.05), but egg mass and feed conversion ratio were higher in the 0.5% group. Triglycerides and cholesterol decreased in the egg yolk and blood serum of birds fed 0.5% TP comparing control birds (p<0.05). At a temperature of 25 °C, the use of 0.5% TP compared to the control treatment increased the Haugh unit but decreased the albumin pH compared to the control treatment and 0.25% TP (p<0.05). Laying hens fed a diet containing 0.5% TP had larger white follicle numbers than birds fed a control diet and 0.25% TP groups (p<0.05). In conclusion, 0.5% TP supplement in the diet of laying hens can increase performance and the number of large white follicles and decrease the level of triglycerides and cholesterol in egg yolk and blood serum.

Keywords: follicle; laying hens; ovary; thyme; yolk lipids

INTRODUCTION

In the animal husbandry and poultry sectors, antibiotics are frequently used to promote growth and production and as prophylactic measures (Diaz-Sanchez *et al.*, 2015). However, the use of antibiotics as a feed supplement has been extensively prohibited due to antibiotic resistance and health concerns associated with antibiotic residues in poultry products (El-Sabrout *et al.*, 2023). Many feed additives, such as prebiotics, probiotics, organic acids, phytogenic compounds, and aromatic plant extracts, are now used as alternative feed additives in poultry production to improve health and performance (Palamidi *et al.*, 2023; El-Sabrout *et al.*, 2023). Many studies have been conducted on using aromatic herbs as feed additives to substitute antibiotics. Livestock feed intake and utilization may be improved by phytogenic additions (Su *et al.*, 2021). They have antimicrobial, antioxidative, and immunomodulatory effects when they are included in livestock diets (Yalcin *et al.*, 2020). Recent studies have reported that medicinal plants improve performance in aged laying hens through phytoestrogenic chemicals (Saleh *et al.*, 2019).

Thyme (*Thymus vulgaris*) is an aromatic plant from the mint family. Its principal components are phenols, thymol (40%), and carvacrol (15%), which are the primary antibacterial active ingredients (Dauqan & Abdullah, 2017). Thyme powder (TP) has been shown to have various therapeutic effects, including antioxidant, antibacterial, anticancer, antiviral, anti-inflammatory, and growth-promoting properties (Abd El-Hack *et al.*, 2016). TP has also been traditionally used for a variety of therapeutic uses, including respiratory illnesses, antimicrobials, and analgesics (Dauqan and Abdullah, 2017). TP contains vitamin B complex, folic acid, β-carotene, vitamins A, K, E, and C, as well as minerals like potassium, calcium, iron, manganese, magnesium, and selenium (Dauqan and Abdullah, 2017). Hashemipour *et al.* (2013) found that phytogenic products containing thymol and carvacrol improve broiler performance, digestive enzyme activity, antioxidant enzyme activity, and immunological response while delaying lipid oxidation. However, only a few studies have been undertaken to explore TP's effects on laying hens' performance, and the findings are highly inconsistent. For example, a recent study found that TP did not influence egg quality features but did considerably improve egg production (EP) and feed conversion ratio (FCR) (Mohammed *et al.*, 2022). In a related study, adding 0, 1, and 2% TP to the diet

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of 36-week-old laying hens had no influence on feed intake (FI), body weight, EP, egg weight (EW), or egg quality parameters (Yalcin *et al.*, 2020). In another study, thyme essential oil increased EP, EW, and egg quality features (Ghanima *et al.*, 2020). According to Gu *et al.* (2021), the resistance of laying hens to oxidative stress declines as they get older. Sufficient antioxidants at this phase can reduce stress on birds and preserve egg quality throughout storage (Mirghelenj *et al.*, 2017a,b; Skřivan *et al.*, 2010). Eggs' interior quality deteriorates significantly, especially at room temperature (Mirghelenj *et al.*, 2017ab). Therefore, it is likely that using TP, due to its antioxidant properties, can help neutralize free radicals and thus protect cells from oxidative damage (Fernandes *et al.*, 2023). Antioxidant sources can also prevent further oxidation of fatty acids in eggs (Rouhanipour *et al.*, 2022).

It was hypothesized that TP could increase egg storage time through antioxidant effects and bioactive compounds, reduce egg cholesterol, and improve egg production. Therefore, considering that the effects of TP on the reproductive system and maintaining the internal quality of eggs during the period of keeping eggs at room temperature have not been investigated, and studies have reported conflicting results on the effect of TP on the performance of older laying hens. This study aimed to examine the effect of adding TP to the diet on performance, blood and egg yolk lipids, reproductive system, and maintaining the internal quality of eggs at different storage temperatures in old laying hens.

MATERIALS AND METHODS

Animal Ethics

This study was approved by the Animal Care Committee and Animal Research Ethics Board from the Department of Animal Science, Urmia University, Urmia, Iran (Approval No: IR.UU.D.REC.1401.058).

Birds and Experimental Treatments

This research selected 144 laying hens (Hy-line-W36), aged 65 weeks, with similar body weight (1570 ± 50 g) and randomly assigned to 3 dietary treatments with 6 replications and 8 birds in each. In order to adapt to the experimental conditions, the birds were first fed with basal diet for 2 weeks and then fed with experimental diets for 8 weeks (56 days). Experimental diets were corn-soy based and included 0%, 0.25%, and 0.5% TP. The TP (without applying any extraction, including oil extraction) was purchased from the Darvash Giah Khazar Medicinal Herbs Complex Company. Analysis of bioactive compounds was performed based on the INSO16019-2 method before the farm experiments began (Table 1). All diets were formulated according to the nutritional recommendations of the Hy-line-W36 (2021) strain (Table 2). The birds were fed the control treatment for two weeks as an acclimation period. The lighting schedule was set as 14 hours of lightning with a light intensity of 30 lux and 10 hours of dark with a light intensity of 3 lux.

Performance

To evaluate the performance indicators of laying hens, eggs were collected twice a day (at 10 a.m. and 4 p.m.). The EP and mean EW were measured (using a 0.01g electronic scale) daily, and the egg mass (EM), FCR, and FI were calculated weekly throughout the experiment. To calculate EM, the average weight of eggs produced per day was multiplied by the percentage of daily EP. The FCR of each experimental unit was calculated by dividing FI by EM (Mosayyeb Zadeh *et al.*, 2023).

Egg Quality Analyses

At the end of the experimental period (8 weeks), two eggs were collected from each replication and kept at room temperature (25 °C) and refrigerator (4 °C) for 30 days. Then, the most important internal quality traits of eggs, including albumen height (AH), Haugh unit (HU), yolk height (YH), yolk index (YI), as well as pH of white and yolk, were measured. The yolk diameter (YD) was measured with a digital caliper (0.01 mm; BakingWin, China) to evaluate the YI, and to evaluate the YI, and the YH was divided by the YD. The egg AH was measured using a manual Haugh Micrometer (Analog Baxlo Haugh Micrometer). So that after breaking the eggs, the whites were placed on a flat surface. Then, the AH and YH values were recorded at every point where the tip of the height gauge touched the egg white (1 cm around the yolk) or the yolk. The following equation was used to calculate the HU (Haugh, 1937):

(1) $HU = \log (AH - 1.7\ EW^{0.37} + 7.57)$

HU was Haugh unit, AH was egg albumen height (mm), EW was egg weight (g), and logarithm based on 10.

To measure the pH of yolk and egg albumen, 2 g of egg albumen and egg yolk were mixed with distilled water at a ratio of 1:9 and stirred well until foam was formed (5 minutes). The pH value was recorded after subsiding the foam produced by inserting a pH meter sensor (Microcontroller MTT 65 model).

Table 1. Some bioactive compounds in the test ingredient of the present study (*Thymus vulgaris*)

Items	Value (mg/kg)
Thymol	46.2
α-Pinene	59.8
α-Thujene	12.3
Camphene	52.2
β-Pinene	63.5
Sabinene	23.7
ρ-cymene	41.6
γ-terpinene	12.9
Linalool	48.1
Camphor	37.2

Note: Number of samples and times tested 10 times (n=10).

Table 2. Ingredients and calculated analysis of layer basal diet

Feed ingredients (% as fed)	%
Corn grain	60.16
Soybean meal	23.01
Wheat bran	3.36
Soy oil	1.47
Dicalcium phosphate	1.36
Oyster shells	9.52
Common salt	0.22
Sodium bicarbonate	0.10
Dl-Methionine	0.19
L-Lysine HCL	0.01
Vitamin premix	0.30
Mineral premix	0.30
Nutrients	Calculated composition
ME (kcal/kg)	2700
Crude protein (%)	16.50
Calcium (%)	4.00
Available phosphorus	0.38
Methionine (%)	0.45
Met+Cys (%)	0.72
Lysine (%)	0.79
Threonine (%)	0.58
Tryptophan (%)	0.22
DEB (Meq/kg)	204

Note: Supplied vitamins per kilogram of diet: Vit A: 10000 IU; Vit D3: 2500 IU; Vit E: 10 IU; Vit B1: 2.2 mg; Vit B2: 4 mg; Vit B3: 8 mg; Vit B6: 2 mg; Vit B9: 0.56 mg; Vit B12: 0.015 mg; Choline 200 mg. Supplied minerals per kilogram of diet: Mn: 80 mg; Fe: 50 mg; Zn: 60 mg; Cu: 12 mg; Sodium Selenite: 0.3 mg.
DEB: Dietary electrolyte balance.

Blood and Egg Yolk Biochemicals Analyses

Two serum samples from each replicate (12 samples per treatment) were prepared at the end of the experimental period to evaluate blood parameters. Blood samples were obtained from the wing vein, and serum samples were collected by placing 5 cc syringes containing blood at an angle at room temperature for 6 hours. To obtain better and clearer samples, serum samples were centrifuged for 12 minutes at 5600 rpm. The samples were transferred to the biochemical laboratory at -20 °C. To measure yolk lipids, 100 mg of egg yolk was weighed with a digital balance (0.001 g; model JT3003D, China) and then mixed with 2.5 mL of NaOH solution and neutralized with 2.5 mL of HCl solution (after 24 hours). Serum cholesterol, triglycerides, VLDL, and egg yolk cholesterol and triglycerides were measured at a wavelength of 550 nm using an enzymatic method. Blood biochemical parameters (uric acid, total protein, and albumin), liver enzymes (aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT)), and antioxidant parameters (malondialdehyde (MDA), and total antioxidant capacity (TAC), were tested using an automated analyzer (Autoanalyser; Technicon RA 1000 model, Bayer) and kits provided by Pars Azmoun Company (Mosayyeb Zadeh *et al.*, 2023).

Reproductive Morphology

At the end of the experimental period, two birds from each replicate were weighed and then killed. The reproductive system, including the ovary and oviduct sections (infundibulum, magnum, isthmus, uterus, and vagina) were weighed (Saleh *et al.*, 2021). The oviduct was washed with saline solution (9 g NaCl per liter of distilled water; physiological serum). Then, the ovary and oviduct were placed in 10% formalin solution for 3 days. After being fixed in 10% formalin, the follicles were counted and weighed according to the method of Robinson and Etches (1986). Thus, the follicles were divided into four categories: large yellow follicles with a diameter of more than 10 mm (LYF), small yellow follicles with a diameter between 10 and 5 mm (SYF), large white follicles with a diameter between 5 and 3 mm (LWF) and medium white follicles with a diameter of 3 to 1 mm (MWF). It should be noted that yellow follicles smaller than 5 mm were classified as atretic follicles (Renema *et al.*, 1995). To measure the weight of the stroma and follicles, the total weight of the stroma, including all the follicles on it, the weight of the stroma after removing the LYFs, and the weight of the stroma after eliminating the other follicles until only the stroma remained was measured Finally, the weight of the largest follicle ready for ovulation (F1) was calculated (Ebeid *et al.*, 2008).

Statistical Analysis

Finally, the obtained data were analyzed using SAS 9.2 statistical software (SAS, 2009). The statistical model for analyzing performance data, blood parameters, and the reproductive system was based on the general linear model (GLM) method as follows (Torshizi & Sedaghat, 2023):

(2) $Y_{ij} = \mu + T_i + e_{ij}$

Y_{ij} was the value of each observation, μ was the mean of observations, T_i was the treatment effect, e_{ij} was the experimental error related to observation.

The following statistical model was used to compare the quality of eggs at different temperatures:

(3) $y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \varepsilon_{ijk}$

y_{ijk} was k-th observation related to j-th level of factor B and i-th level of factor A, Ai was effect of i-th level of factor A, B_j was effect of j-th level of factor B, $(AB)_{ij}$ was interaction effect of factor A and B, and ε_{ijk} was experimental error with zero mean and variance. Factor A was the level of thyme powder, and factor B was the storage temperature. The significance of differences between data means was examined using Tukey's test and P-values less than 0.05.

RESULTS

Blood Variables

The results of the effect of different levels of TP on blood variables of laying hens are reported in

Table 3. The results showed that the concentration of total protein, ALT, ALP, and MDA were affected by different levels of TP. The total protein, ALP, and MDA concentration in the 0.5% TP treatment was significantly lower than in the control treatment (p<0.05). ALT concentration in hens fed 0.25% and 0.5% TP was significantly lower than in the control group (p<0.05).

Production Performance

Table 4 shows the effects of different levels of TP on the egg production performance of laying hens. According to the results, EW, EM, and FCR were affected by the TP supplementation. Laying hens fed with diets containing 0.25% and 0.5% TP had more EW than the control group (p<0.05). EM and FCR in the birds fed 0.5% TP were significantly higher and lower than the control birds (p<0.05). The TP supplementation did not affect the EP and FI of birds (p<0.05).

Egg Quality

The effects of TP levels and storage temperature on the internal quality traits of eggs are given in Table 5.

Table 3. Serum biochemicals of laying hens with different levels of thyme plant powder

Thyme (%)	Variables of serum biochemicals							
	Uric acid (mg/dL)	Total protein (g/mL)	Albumin (g/mL)	ALT[1] (IU/L)	AST[2] (IU/L)	ALP[3] (IU/L)	TAC[4] (mmol/L)	MDA[5] (µg/mL)
Control	6.10	6.77[a]	3.24	56.00[a]	259.40	654.00[a]	2.18	2.42[a]
0.25	5.36	6.80[a]	2.90	26.25[b]	285.00	574.00[a]	1.73	1.42[b]
0.50	6.31	5.50[b]	2.60	21.50[b]	272.00	390.00[b]	1.86	1.68[b]
SEM	0.998	0.317	0.327	1.016	9.84	38.41	0.278	0.159
p-value	0.21	0.027	0.411	<0.01	0.51	0.049	0.314	0.010

Note: Means within the same column with different superscripts differ significantly (p<0.05). [1]ALT: Alanine transaminase; [2]AST: Aspartate transaminase; [3]ALP: Alkaline phosphatase; [4]TAC: Total antioxidant capacity; [5]MDA: Malondialdehyde.

Table 4. Production performance of laying hens with different levels of thyme plant powder

Thymes (%)	Variables of production performance						
	Initial weight (g)	Final weight (g)	Egg production (%)	Egg weight (g)	Egg mass (g)	Feed intake (g)	FCR (g: g)
0 (Control)	1576.36	1584.15	68.66	61.95[b]	42.54[b]	98.3	2.31[a]
0.25	1572.08	1581.66	69.08	63.07[a]	43.57[ab]	98.4	2.25[ab]
0.5	1569.47	1578.24	70.53	63.19[a]	44.57[a]	98.1	2.20[b]
SEM	21.93	19.65	0.744	0.273	0.575	0.412	0.033
p-value	0.71	0.84	0.216	0.013	0.050	0.889	0.021

Note: Means within the same column with different superscripts differ significantly (p<0.05). FCR: feed conversion ratio (g feed consumed: g produced egg).

Table 5. Egg quality of laying hens with different levels of thyme plant powder and storage temperature

Treatments	Variables of egg quality				
	HU	YH	YI	YpH	ApH
Main effects of thymes supplementation					
0% thymes (Control)	59.97	15.69[b]	35.75[b]	7.05	9.72[a]
0.25% thymes	66.73	14.24[c]	32.30[c]	6.98	9.31[ab]
0.5% thymes	66.77	17.88[a]	41.24[a]	7.05	8.91[b]
SEM	2.006	0.273	0.620	0.082	0.146
P-value	0.094	<0.01	<0.01	0.76	0.006
Main effects of storage temperature					
4 °C	81.83[a]	18.25[a]	42.15[a]	6.94	9.03[b]
25 °C	47.15[b]	13.62[b]	30.71[b]	7.12	9.53[a]
SEM	1.638	0.223	0.506	0.067	0.119
P-value	<0.01	<0.01	<0.01	0.072	<0.01
Interactions					
0% thymes × 4 °C	78.89[ab]	18.94[b]	43.55[b]	7.05	9.37[ab]
0% thymes × 25 °C	41.05[c]	12.44[d]	27.96[d]	7.05	10.07[a]
0.25% thymes ×4 °C	83.37[a]	14.24[c]	32.66[c]	6.84	8.92[b]
0.25% thymes ×25 °C	50.10[b]	14.24[c]	31.93[c]	7.11	9.70[a]
0.5% thymes ×4 °C	83.23[a]	21.58[a]	50.23[a]	6.92	8.81[b]
0.5% thymes ×25 °C	50.30[b]	14.18[c]	32.25[c]	7.18	9.02[b]
SEM	2.838	0.387	0.877	0.117	0.207
p-value	0.006	<0.01	<0.01	0.44	0.014

Note: Means within the same column with different superscripts differ significantly (p<0.05). HU: Haugh unit; YH: yolk height; YI: yolk index; YpH: yolk pH; ApH: albumen pH.

The results showed that at a temperature of 25 °C, the use of 0.5% TP increased the YH and egg YI compared to the control group (temperature of 25 °C and 0.0% TP) ($p<0.05$). At a temperature of 25 °C, the use of 0.25% and 0.5% TP compared to the control treatment increased the Haugh unit. Storing eggs at room temperature increased albumen pH (0% thymes × 25 °C), but the use of thyme (0.5% thymes × 25 °C) decreased albumen pH ($p<0.05$).

Blood and Egg Yolk Lipids

Table 6 shows the effects of different levels of TP on serum and egg yolk cholesterol and triglyceride. Laying hens fed a diet containing 0.5% TP had lower triglyceride and cholesterol concentrations in egg yolk than in the control group ($p<0.05$). The triglyceride, cholesterol, and VLDL concentration in the 0.5% TP treatment was significantly lower than in the control treatment ($p<0.05$).

Reproductive Morphology

Table 7 shows the results of different levels of TP on the reproductive system and ovarian follicles in laying hens. According to the obtained results, the weight and size of the reproductive organs were not affected by the use of different levels of TP in the diet. Laying hens fed with a diet containing 0.5% TP had a larger white follicle number than the control group and 0.25% TP ($p<0.05$). The weight of the large yellow follicle in the 0.25% TP treatment was significantly higher than the control treatment ($p<0.05$).

DISCUSSION

Blood Parameters

As laying hens age, oxidative stress increases, lowering their immunological and antioxidant capacities, reducing ovarian function, and damaging cells in the reproductive system (Xie *et al.*, 2019). Aged laying hens are susceptible to oxidative stress, which can negatively impact their performance (Gu *et al.*, 2021). According to Lee *et al.* (2004), aging is a normal and irreversible physiological process that might gradually develop dangerous reactive oxygen species. Therefore, oxidative stress and a disturbance of redox homeostasis would unavoidably arise if endogenous antioxidants were unable to counteract the high levels of free radicals and peroxides in the organisms (Estevez, 2015). Decreased cellular volume and the buildup of cytoplasmic lipofuscin, a highly oxidized and cross-linked protein that can cause oxidative stress, are natural alterations in aging hepatocytes (Pinto *et al.*, 2020). Furthermore, it has been demonstrated that the mRNA abundance of genes related to antioxidants

Table 6. Blood and egg yolk lipids of laying hens with different levels of thyme plant powder

Thyme (%)	Yolk		Serum		
	Triglyceride (mg/g)	Cholesterol (mg/g)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	VLDL (mg/dl)
Control	207.20[a]	33.20[a]	2817.60[a]	216.40[a]	563.52[a]
0.25	184.80[b]	29.00[ab]	2268.00[ab]	201.00[a]	453.60[ab]
0.50	185.40[b]	28.20[b]	1372.00[b]	105.00[b]	274.40[b]
SEM	5.004	1.208	302.438	18.027	60.487
p-value	0.027	0.012	0.017	<0.01	0.017

Note: Means within the same column with different superscripts differ significantly ($p<0.05$). VLDL: very low-density lipoprotein; SEM: standard error of the mean.

Table 7. Table 7. Reproductive system morphometry of laying hens with different levels of thyme plant powder

Variables	Treatments			SEM	p-value
	Control	0.25	0.50		
Ovary (%BW)	2.59	3.19	2.79	0.311	0.413
Oviduct (%BW)	4.30	4.51	4.01	0.294	0.508
Infundibulum (%oviduct)	4.37	3.54	3.51	0.452	0.351
Magnum (%oviduct)	51.38	57.27	54.42	2.294	0.371
Isthmus (%oviduct)	5.70	5.59	5.14	0.352	0.497
Uterus (%oviduct)	27.53	26.21	27.53	1.902	0.887
Vagina (%oviduct)	11.29	9.41	6.65	1.197	0.063
LYF number	5.00	5.00	4.00	0.408	0.191
SYF number	10.00	9.25	11.25	0.993	0.393
LWF number	31.25[b]	29.50[b]	44.50[a]	2.190	<0.01
LYF (g)	24.27[b]	35.72[a]	33.15[ab]	2.566	0.027
SYF (g)	1.32	1.35	1.12	0.342	0.88
Stroma (g)	5.87	7.10	5.15	0.627	0.139
F1 (g)	13.62	15.00	15.25	0.706	0.200

Note: Means within the same column with different superscripts differ significantly ($p<0.05$). BW: body weight; LYF: large yellow follicle; SYF: small yellow follicle; LWF: large white follicle; F1: the largest follicle ready for ovulation.

changes from the prenatal stage to adulthood (60 days) in mice. This suggests that the liver may contain an age-dependent pattern of antioxidant genes (Wu *et al.*, 2019), and certain changes may also be anticipated as the animals reach their senior years. By preventing the free radical chain reaction from damaging the cell membrane, antioxidants can slow the aging process of the body and reproductive system (Miyazawa *et al.*, 2019). The use of medicinal plants in poultry nutrition has increased due to their positive effects on blood parameters, immune system, antioxidant capacity, and, ultimately, bird performance (Pliego *et al.*, 2022).

The addition of TP in the diet of laying hens significantly decreased the activity of ALT and ALP. The results show the positive effect of TP in improving liver function, which is consistent with the results of Tekce and Gül (2017). These researchers reported that using medicinal plants such as *Origanum syriacum* containing carvacrol and thymol in the diet of laying hens reduces the levels of liver enzymes (ALT and AST). Researchers showed that medicinal plants regulate the activity of these enzymes by suppressing liver enzymes (Fakhri *et al.*, 2022). Therefore, adding TP to the diet of laying hens due to its antioxidant properties can protect cells from DNA damage and thus benefit the health of laying hens. It is assumed that the antioxidant property of TP protects lipids from oxidation and thus delays the process of lipid peroxidation (Büyükkılıç *et al.*, 2020). In agreement with our results, Mosayyeb Zadeh *et al.* (2023) showed that the diet significantly reduced MDA by adding pennyroyal supplementation (0.5% or 1%). TP helps in antioxidant activity because it reduces the level of MDA (the most important indicator of fat in any oxidation). Also, Gumus *et al.* (2017) stated that extracts significantly reduced MDA levels. Dietary extracts such as carvacrol and thymol can initiate excessive free radicals due to their phenolic OH groups as hydrogen donors for peroxy radicals produced during lipid oxidation, thereby reducing hydroxyl peroxide formation (Gavaric *et al.*, 2015). Based on these results, we demonstrated that TP may play a major role as an exogenous antioxidant and can also be used as a protective agent against tissue damage.

Production Performance

The results of the present study showed that the use of 0.5% TP in the diet of laying hens significantly increased EW and EM. FCR is an indicator that depends on EM and FI. Based on this, it is believed that the increase in EM (while the feed intake remained unchanged) during the study may have led to a decrease in the FCR. Therefore, any factor that increases EW may affect these parameters. In agreement with our results, Mohammed *et al*. (2022) reported that feeding laying hens with 5 and 10 g of TP per kg of feed improved EM and FCR compared to the control treatment. Some previous studies have shown that TP or thyme extract improves EP parameters (Abd El-Hack & Alagawany, 2015; Abdel-Wareth, 2016).

On the other hand, Yasin *et al.* (2025) showed that a diet containing 2.5% TP had a negative effect on the productive performance of laying hens. Still, in agreement with the results of the present study, several studies reported the lack of effect of TP on the percentage of EP (Bala *et al.*, 2021). The improvement in EW may be attributed to an increase in the digestibility of dietary nutrients and digestive capacity, resulting in better availability of these nutrients (protein, amino acids, and minerals) in the small intestine (Windisch *et al.*, 2008). Olgun (2016) reported that thyme essential oil may improve ovarian function and digestibility of nutrients in the intestine, thereby increasing EW and EM in laying hens. Thymol protects intestinal microvilli involved in nutrient absorption and affects the secretion of endogenous digestible enzymes (Hashemipour *et al.*, 2013). Extracts or powders of medicinal plants have an essential effect on FCR, as they contribute to the stability of the microorganism population and increase nutrient uptake (Gholami-Ahangaran *et al.*, 2022). In addition, these compounds can activate digestive enzymes and improve the digestion of nutrients (Youssef *et al.*, 2021). Galli *et al.* (2020) found that carvacrol and thymol improved FCR in broilers by inactivating insulin sites in the liver. Also, carvacrol and thymol help protein digestion by increasing the secretion of pepsin and hydrogen chloride (Li *et al.*, 2023). Some researchers investigated the effect of thymol at 100 ppm in the diet on enzyme activity in broiler chickens and observed a 29% increase in lipase and an 18% increase in trypsin activity in the digestive system (Galli *et al.*, 2020). In another study, Recoules *et al.* (2017) reported a significant increase in the activity of pancreatic amylase, maltase, and trypsin in broilers fed with a mixture of plant extracts. In this study, the positive effect of TP on EW, EM, and FCR can be attributed to the improvement of digestion and absorption of nutrients and the overall health of the digestive system (Shahryar *et al.*, 2011). The active ingredients in TP (thymol and carvacrol) strengthen digestive enzymes such as amylase, protease, and lipase, resulting in better digestion of nutrients and improved absorption (Mohammed *et al.*, 2022). Also, in this experiment, the increase in EW without increasing the FI can be caused by the beneficial effects of the compounds in TP. TP contains compounds such as carvacrol and menthol, which have antimicrobial properties and make the digestive tract's environment healthy. They prevent the breakdown of amino acids by harmful microbes. Increasing the surface and number of intestinal finger cells provides the basis for absorbing more nutrients (Torki *et al.*, 2021). Various experiments have been conducted to prove the effect of TP and thyme extract on poultry performance, and sometimes contradictory results have been obtained; for example, a positive effect on performance has been reported in laying hens (Ghanima *et al.*, 2020). On the other hand, there are reports that TP has no effect on the performance of laying hens (Ding *et al.*, 2017; Olgun & Yıldız, 2014). The comparison of the studies shows that there may be differences in the chemical composition of the active ingredient and their amount in TP, the type of basic diet, the age of the laying hens, and the way of use (in the form of powder or extract) or the different levels used in different researches can be the reason for the contradiction in the results of different experiments.

Egg Quality

The evaluation of egg quality is based on two factors: the external quality that can be seen from external observation and the internal quality that may be determined after breaking the egg. The internal quality of eggs starts to decline immediately after laying, depending on the eggs' movement and the conditions of keeping, managing, and feeding the birds. Among the various factors affecting egg quality, temperature, humidity, and the presence of carbon dioxide are vital (Abebe *et al.*, 2023). Genetics and environmental factors like storage temperature, time, and humidity affect the quality of egg albumen, or HU, which is regarded as a standard criterion for determining the freshness of eggs in the poultry business (Wang *et al.*, 2019). In the present study, egg storage at 25 °C compared to 4 °C caused a decrease in egg AH and HU and an increase in egg albumen pH. According to reports, the albumen thins and liquefies over time, eventually becoming a thin white substance (Kumari *et al.*, 2020). As is well known, this causes the carbonic acid in egg albumen to break down into carbon dioxide and water, which lowers the HU of eggs while they are being stored. The evaporation of carbon dioxide and water through the pores of the eggshell leads to an increase in the pH of the albumen to an alkaline state because the egg albumen loses its gel structure, which ultimately leads to a decrease in the HU (Yimenu *et al.*, 2017). According to Nematinia and Abdanan Mehdizadeh (2018), the pH of fresh egg albumen is approximately 7.6, and it rises during storage due to carbon dioxide being lost through shell pores (Marzec *et al.*, 2019). At higher temperatures, a faster loss of carbon dioxide leads to a faster deterioration of the albumen quality (Kumari *et al.*, 2020). Ovomucin, a protein found in egg albumen, plays a major role in creating the gel-like consistency of egg albumen. During storage, due to the loss of carbon dioxide and the increase in the pH of the egg albumen, the instability of the ovomucin protein complex and the breaking of some disulfide bonds between the egg albumen proteins occur, which leads to a decrease in the AH and the HU (Kumari *et al.*, 2020). Another variable that was affected by the storage temperature was YI and YH. The yolk of freshly laid eggs is round and hard (Zhang *et al.*, 2019). As mentioned, during the storage of eggs at high temperatures, carbonic acid is decomposed into carbon dioxide and water. Then, water enters from the albumen to the yolk through the vitelline membrane (Luo *et al.*, 2020) until the concentration (pressure) between the two phases (i.e., egg albumen and yolk) becomes the same, and this leads to the swelling of the yolk, which in turn weakens and stretches the vitelline membrane (Brodacki *et al.*, 2019). This pressure eventually causes the yolk to turn from a round shape into a loose and spherical mass (Caner & Yuceer, 2015), and the YH and YI decrease.

The main effects of TP level and the interaction effect of TP level and storage temperature showed that the use of 0.5% TP, especially at 4 °C, prevents the drop of YH, YI, and egg albumen pH. According to Ashour *et al.* (2014), using medicinal herbs or their extracts causes MDA levels to drop and some antioxidant enzymes, such as glutathione peroxidase, to rise. Therefore, raising glutathione peroxidase levels may aid in preserving laying hens' antioxidant systems. The high biological activity of TP as a natural antioxidant is thought to be caused by the presence of phenolic hydroxy compounds, which serve as hydrogen donors to the proxy radicals generated in the initial stage of lipid oxidation (Hashemipour *et al.*, 2013). Büyükkılıç *et al.* (2020) found that adding TP to the diet of laying hens prevents lipid oxidation in refrigerated eggs. In several other studies (Reis *et al.*, 2019; Mirghelenj *et al.*, 2017a), the use of 30 g and 4.5% grape pomace in the diet of laying hens decreased yolk and albumen pH, reduced egg fat oxidation, decreased caproic content, butyric and margaric fatty acids, and increased HU. On the other hand, Goliomytis *et al.* (2018) used dry orange pomace in the amount of 90 g/kg in the diet of laying hens. They observed a significant increase in the oxidative stability of egg yolk. Therefore, the use of TP as a herbal additive and natural antioxidant can probably maintain the internal quality of eggs during storage at different temperatures to some extent.

Blood and Egg Yolk Lipids

In agreement with the results of the present study, serum cholesterol and triglyceride levels in the study of Mohammed *et al.* (2022) of chickens treated with TP were lower compared to control hens. According to Saadat Shad *et al.* (2016), carvacrol and thymol are responsible for the decrease in serum cholesterol levels. Bayatmakoo *et al.* (2017) showed that thymol may exert hypocholesterolemic effects by inhibiting coenzyme 3-hydroxy-3-methylglutaryl A reductase. The reduction of serum cholesterol by TP may explain the reduction of MDA in birds fed with that diet. Ding *et al.* (2022) reported that using medicinal plants containing phenolic compounds in poultry nutrition can disrupt the absorption and reabsorption processes by disrupting the lipogenesis process. This hypothesis was confirmed by Torki *et al.* (2018), as these researchers showed that the phenolic compounds of medicinal plants, possibly by binding bile acids, prevent their absorption and ultimately disrupt the entero-hepatic cycle.

Reproductive Morphology

The present experiment assumed that TP-containing estrogenic compounds (Zhang *et al.*, 2024) can effectively affect steroid hormones in folliculogenesis. In this connection, it has been reported that adding phytoestrogen sources to the diet of laying hens improves antioxidant status, hormonal profile, and steroidogenesis in aged laying hens (Saleh *et al.*, 2019). However, the results of the present study show that TP had a significant effect, as was also reported in the study of Saki *et al.* (2014), who used a mixture of medicinal plants. Research also found that fennel essential oil significantly increases the weight of large yellow follicles and their number (Taki *et al.*, 2014). Saleh *et al.* (2019) confirmed the direct relationship between dietary plant composition and blood estradiol-17β levels in older laying hens, following previous studies (Obianwuna *et al.*, 2024), and concluded that the improvement of performance in birds fed with flaxseed and fenugreek may be due to the increase of estradiol-17β levels in the blood due to the regulation of estrogen receptor-β mRNA in liver cells.

CONCLUSION

In general, adding 0.5% of thyme plant powder to the diet of laying hens can improve egg weight, FCR, and ovarian indices and prolong egg storage time. Also, thyme decreases serum cholesterol, triglycerides, liver enzymes, egg yolk cholesterol, and triglycerides. Finally, thyme proved to be a promising feed additive for feeding laying hens.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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