Research Article

# Effect of *Thymus vulgmus* Addition to the Diet of Laying Hens on Egg Production, Egg Quality, Biochemical and Antioxidant Parameters

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**Abstract** | This study evaluated the impact of thyme powder supplementation on the hen egg production (HD%), total egg production, egg quality, antioxidant and biochemical parameters of laying hens. One hundred and eighty Hy-line Brown hens, 40 weeks old were randomly divided into 9 groups of 20 hens and subjected to one of the following treatments: $T_1$ (control diet), $T_2$ (5g/kg of thyme), and $T_3$ (10g/kg of thyme). Each treatment was tested using three groups of hens, which were subject to treatments from 40 to 47 weeks of age. The productive metrics were measured on daily and weekly basis in the period from 40 to 47 weeks of age, while the egg quality was measured after 56 days. Hematological, biochemical and antioxidants parameters were also determined at the end of the experiment. The results showed that the egg production and feed conversion ration were significantly improved in both thyme treatments ($T_2$ and $T_3$). Also, the PCV (%) and the WBC count. However, there was no significant difference in the egg quality between the thyme-treated and control hens. The hens on thyme-supplemented diets were found to have lower serum cholesterol concentration than those of the control. Supplementing a laying hen's diet with thyme significantly increased glutathione, while, decreased the malondialdehyde, AST and ALT activity in comparison to the control. Therefore, it can be concluded that thyme additives can be used in laying hens diet to improve egg production, egg quality, antioxidant and biochemical parameters in a dose-dependent manner.

**Keywords** | Antioxidants, Egg production, Laying hens, Thyme

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## INTRODUCTION

In Iraq, commercial breeders of laying hens have a goal of increasing the number of eggs produced per unit of time. Nutrition is vital issue for the optimal care and handling of poultry as a has direct impact on egg production and poultry health (Dilawar et al., 2021). Antibiotic-free diets are the most important factor in the laying hen industry due to the increasing customers interest in antibiotic-free products, this led to an intensification in research for the development of effective antibiotic alternatives. This resulted in the appearance of plant-based feed additives that can be used to improve the growth, quality and health of poultry. Medicinal plants are considered to be a suitable antimicrobial alternative (Bozkurt et al., 2016).

Previous studies showed that the active components of medicinal plants can improve poultry health and growth. It has also been discovered that using plant-based compounds in poultry diets increases productivity and immunity (Harrington et al., 2019). Thyme (*Thymus vulgaris*) is a versatile herb belongs to *Lamiaceae* family, used in both fresh and dried forms for various purposes, and its essential oil is extracted and mixed with those of other aromatic herbs. The chemical composition of thyme is 40% thymol and 15% carvacrol (Mikaili, 2010). The essential oils from rosemary, oregano, and thyme have been found to improve egg production of laying hens (Cimrin, 2019). It has been also reported that thyme have a good antimicrobial and antioxidant activities. These biological properties are also known to improve chicken health (Alagawany and Abd El-Hack, 2015). Thymol is a positive allosteric modulator that can stimulate the activity of GABA receptors (García et al., 2006). It can also be used as a drug to reduce pain (Bhandari and Kabra, 2014). The consumption of a combination of thyme, mint, and rosemary can improve the productivity and health of hens. It can also decrease malondialdehyde, increase glutathione (GSH) in the liver, and increase superoxide dismutase (SOD) activity (Abd El-Hack and Alagawany, 2015). Extracts from such aromatic plants were added also to the hen's diet to enhance their antioxidant levels (Świątkiewicz et al., 2018). Bölükbaşi and Erhan (2007) and Abdel-Wareth (2016) have shown that thyme additives improve egg-quality and performance of hens. Although some studies haven't found a significant impact of various herb supplementation on egg -production (Arpášová et al., 2014; Ding et al., 2017).

Hence, further studies are required to resolve these discrepancies. This investigation was to evaluate the usefulness of thyme addition to laying hens' diet on productivity, egg quality, biochemical and antioxidant parameters.

## MATERIALS AND METHODS

### Birds and experimental design

This research project was conducted at the laying hen farm within the Department of Animal Production at Tikrit University's, College of Agriculture, following guidelines for the use of animal that were approved by the Institutional Animal-Ethic Committee (No.AS-3002P).

One hundred and eighty Hy-line Brown hens, 40 weeks old were randomly allocated in 9 groups (20 hens, each), where three groups were assigned to each of the three different feeding treatments that was applied from 40 to 47 weeks of age. Hens were *fed ad libitum* a commercial layer diet (NRC, 1994) consists of Me (kcal kg-1) 2800, Crude protein (15.77%) and Calcium (3.58%), the diet composition are shown in Table 1. The treatments diet was, ($T_1$) Control treatment; ($T_2$) 5 g/kg of thyme powder and ($T_3$)10 g/kg of thyme powder.

### Egg production and quality

The hen egg production (HD%) was measured for each treatment and cumulative egg production on weekly basis was also calculated during the period from 40-47 weeks of age. The average egg weight was multiplied by the HD% rate to calculate the egg mass. By dividing the feed intake on the egg mass, the feed conversion ratio (FCR) was calculated. Six eggs were randomly chosen from each group (a total of eighteen per treatment) for measurement of egg quality and was assessed on the inside and outside at day 56. An electronic scale was used to weight the eggs, and a strength tester to measure the eggshells after broken the eggs carefully on a plate (glass). The egg's yolk was separated from the albumin. Yolk and albumin weight was determined by an electronic scale. Yolk height was measured by a vernier calliper. A Haugh unit (HU) was calculated using egg white protein (albumin) measurements, and the eggshell thickness using a digital micrometer, as described by Haugh (North, 1984). The width of the egg was divided on the egg length to get the index of the egg shape.

**Table 1:** Component's composition of laying hens' diet.

| Components | % |
|---|---|
| Corn | 40.5 |
| Wheat | 30 |
| Soybean meal | 17.85 |
| Premix[a] | 2.5 |
| Oil | 0.55 |
| Oyster shell | 8.6 |
| | 100 |
| ME (kcal kg-1) | 2800 |
| Crude Protein (%) | 15.77 |
| P available (%) | 0.50 |
| Lys. (%) | 0.76 |
| Meth. (%) | 0.40 |
| Methionine + cysteine (%) | 0.66 |
| Ca (%) | 3.58 |

[a] The Premix (1 kilogram of Premix): vit. E, 500 IU; vit. $B_{12}$, 0.06 mg; vit. $B_1$, 67 mg; vit. A, 334000 IU; vit. $D_3$, 67000 IU; vit. $B_2$, 1000 mg; vit. B6, 0.66 mg; folic acid, 17 mg; choline, 17000 mg; N., 1000 mg; Mg, 3.334 mg; Zinc, 334 mg; Iron, 1.667 mg; Cop., 10 mg; I, 17 mg; Meth., 27000 mg; Phosphor, 10.6% and Se, 0.20 mg.

### Blood sampling

Nine birds from each group (three hens each replicate) were randomly selected at day 56 (end of the experiment). A blood sample was collected from the wing vein of the hens into two separate vials (5 mL each). The first vial contained heparin to help determine hematological parameters and separate the plasma, while the second did not contain any anticoagulant to obtain serum. The samples were centrifuged at 4000 rpm for 10 min, plasma and serum were separated and stored at –20 °C until analysis.

### Hematological parameters

Red blood cell (RBC) and white blood cell (WBC) counts were measured according to (Natt and Herrick, 1952). The packed cell volume (PCV) was determined according to (Dacie and Lewis, 1975).

### Biochemical and antioxidants parameters

Serum cholesterol level was calorimetrically determined according to (Kattermann, 1979), and the plasma triglyceride level was determined by the enzymatic method (Sigma Diagnostics Kit No. 339). Also, plasma samples were analyzed for total protein by the Biuret method according to (Yousef and El-Demerdash, 2006); and Albumin concentration calorimetrically according to (Doumas et al., 1971), Globulin concentration was estimated by separation of albumin from total protein. Glucose was estimated in the serum by an enzymatic method (Biosystems SA Kit, No. REF11533). To estimate liver function, ALT and AST activity was calorimetrically determined according to (White et al., 1970). GSH glutathione peroxidase activity (Günzler et al., 1974) and the malondialdehyde concentration were determined according to (Romero et al., 1998).

### Statistical analysis

Data were analyzed by one-way ANOVA using the statistical program SAS (Ver. 9.0). Duncan's test was employed for multiple comparisons. $p < 0.05$ were used to identify key differences between the treatments.

## RESULTS AND DISCUSSION

### Egg production

The egg production data of laying hens were given in Table 2. A significant increase was observed in hen egg production (HD%), egg mass, cumulative egg number and egg weight in thyme-treated when compared to control hens throughout the period from 40-47 weeks of age and as overall. Also, a significant ($p < 0.05$) improved was found in the FCR of the thyme-treated hens during the experimental period.

### Egg quality

Table 3 showed that there was no significant effect for thyme addition on most of measured parameters of egg quality ($p > 0.05$), except for the increase in albumin index (%) and eggshell index (%).

### Hematological parameters

The hematological results were summarized in Table 4. There was no significant ($p > 0.05$) difference in RBC values in treatments. However, PCV (%) showed a significant increase in the $T_3$ hens compared with control. The WBC count significantly increased in response to both thyme treatment in contrast with the control.

### Biochemical serum parameters

Figure 1 showed the serum biochemical parameters. Figure 1a showed that thyme-treated hens had non-significant difference in serum glucose level ($p>0.05$) between treatments. The albumin and total protein levels showed no difference among all treatments ($p>0.05$) (Figure 1b, c). However, the hens in the thyme treatments showed improved globulin values (Figure 1d).

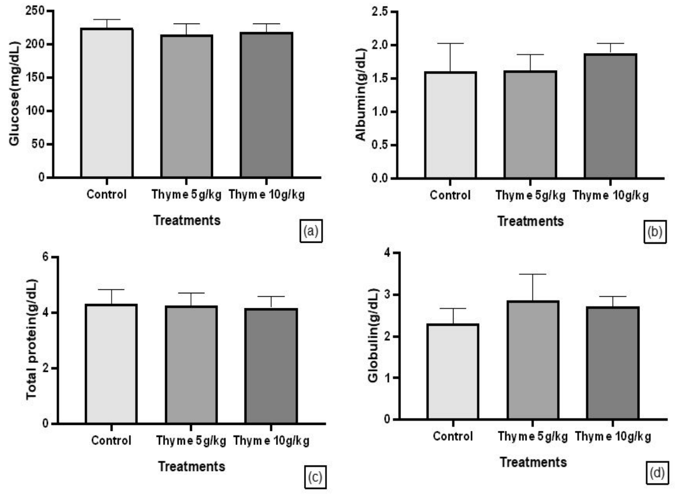


**Figure 1:** Blood parameters in the serum of laying hens fed 5 and 10 g/kg of thyme powder during the 56-day study period: (a) Glucose, (b) Albumin, (c) Total protein and (d) Globulin.

### Lipid profile

The effects of thyme additives on the hen lipid profile and liver function were presented in Table 5. Throughout the 56-day study period, plasma triglyceride concentration was not significantly affected by thyme powder treatment. The thyme treatment decreased significantly ($p < 0.05$) the serum cholesterol concentration. The thyme-treated hens showed the lowest ALT and AST activity compared with the control hens throughout the 56-day study period.

*Antioxidant parameters*

Figure 2a showed a significant increase (p < 0.05) in the GSH (nmol/mL) levels in the thyme-treated hens compared with the control hens. However, the thyme-treated hens had lower MDA (nmol/mL) levels than the control hens (Figure 2b).

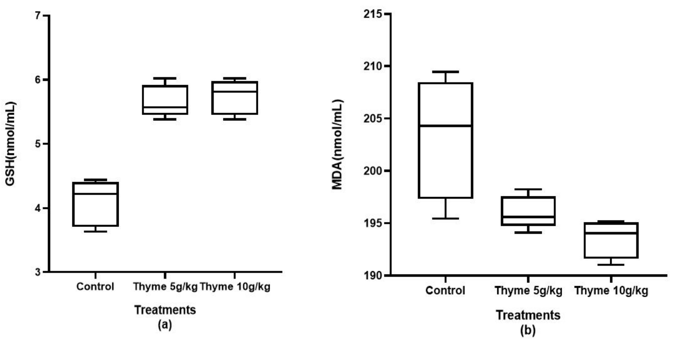


**Figure 2:** Oxidative parameters in the serum of laying hens fed 5 and 10 g/kg of thyme powder during the 56-day study period: (a) GSH and (b) MDA.

**Table 2:** Influence of thyme powder (*Thymus vulgaris*) on the productivity of hens from (40-47 weeks) of age.

| Treatments | Parameters | | | | |
|---|---|---|---|---|---|
| | HD (%) | FCR | Cumulative egg number | Egg weight (g) | Egg Mass (g/h/d) |
| **Week 40** | | | | | |
| $T_1$ (Control) | 94.64 ± 0.35[a] | 2.81 ± 0.01[a] | 6.62 ± 0.02[a] | 55.67 ± 0.52[a] | 52.69 ± 0.30[a] |
| $T_2$ (5g.kg) | 95.71 ± 0.71[a] | 1.97 ± 0.01[b] | 6.70 ± 0.05[a] | 60.92 ± 0.07[a] | 58.31 ± 0.50[a] |
| $T_3$ (10g.kg) | 95.71 ± 1.42[a] | 1.97 ± 0.02[b] | 6.70 ± 0.12[a] | 61.08 ± 0.53[a] | 58.46 ± 0.58[a] |
| **Week 41** | | | | | |
| $T_1$ (Control) | 88.92 ± 3.21[b] | 2.28 ± 0.09[a] | 6.22 ± 0.22[a] | 56.83 ± 0.23[b] | 50.53 ± 2.03[b] |
| $T_2$ (5g.kg) | 95.36 ± 2.50[a] | 1.92 ± 0.05[b] | 6.67 ± 0.17[a] | 62.84 ± 0.04[a] | 59.92 ± 1.53[a] |
| $T_3$ (10g.kg) | 96.42 ± 0.71[a] | 1.95 ± 0.04[b] | 6.75 ± 0.05[a] | 61.10 ± 0.86[a] | 58.91 ± 1.27[a] |
| **Week 42** | | | | | |
| $T_1$ (Control) | 92.14 ± 2.85[b] | 2.17 ± 0.06[a] | 6.45 ± 0.20[a] | 57.34 ± 0.06[b] | 52.83 ± 1.58[b] |
| $T_2$ (5g.kg) | 95.35 ± 3.21[a] | 1.86 ± 0.05[b] | 6.67 ± 0.22[a] | 64.81 ± 0.51[a] | 61.78 ± 1.59[ab] |
| $T_3$ (10g.kg) | 93.57 ± 1.43[a] | 1.98 ± 0.02[b] | 6.55 ± 0.10[a] | 61.97 ± 0.25[a] | 57.99 ± 0.65[a] |
| **Week 43** | | | | | |
| $T_1$ (Control) | 90.71 ± 3.57[b] | 2.10 ± 0.09[a] | 6.35 ± 0.25[b] | 60.37 ± 0.20[a] | 54.77 ± 2.34[b] |
| $T_2$ (5g.kg) | 92.86 ± 1.43[a] | 1.90 ± 0.02[b] | 6.50 ± 0.10[a] | 65.20 ± 0.30[a] | 60.54 ± 0.65[a] |
| $T_3$ (10g.kg) | 91.78 ±1.07[a] | 1.95 ± 0.04[b] | 6.42 ± 0.07[a] | 64.11 ±0.72[a] | 58.85 ± 1.35[a] |
| **Week 44** | | | | | |
| $T_1$ (Control) | 87.86 ± 3.57[b] | 2.15 ± 0.05[a] | 6.15 ± 0.10[a] | 60.84 ± 0.44[b] | 53.46 ± 1.26[b] |
| $T_2$ (5g.kg) | 90.71 ± 1.42[a] | 1.93 ± 0.01[b] | 6.35 ± 0.10[a] | 65.55 ± 0.40[a] | 59.46 ± 0.57[a] |
| $T_3$ (10g.kg) | 91.78 ± 1.07[a] | 1.95 ± 0.04[b] | 6.42 ± 0.12[a] | 64.30 ± 0.15[a] | 59.01 ± 1.28[a] |
| **Week 45** | | | | | |
| $T_1$ (Control) | 78.93 ± 1.07[b] | 2.39 ± 0.01[a] | 5.52 ± 0.07[b] | 60.92 ± 0.12[b] | 48.09 ± 0.75[b] |
| $T_2$ (5g.kg) | 89.64 ± 1.78[a] | 1.94 ± 0.04[b] | 6.27 ± 0.12[a] | 65.95 ± 0.10[a] | 59.12 ± 1.26[a] |
| $T_3$ (10g.kg) | 90.35 ± 0.35[a] | 1.94 ± 0.05[b] | 6.32 ± 0.02[a] | 65.56 ± 0.46[a] | 59.23 ± 0.19[a] |
| **Week 46** | | | | | |
| $T_1$ (Control) | 76.43 ± 2.14[b] | 2.44 ± 0.07[a] | 5.35 ± 0.12[b] | 61.77 ± 0.07[b] | 47.22 ± 1.38[b] |
| $T_2$ (5g.kg) | 90.35 ± 0.35[a] | 1.90 ± 0.01[b] | 6.32 ± 0.25[a] | 66.83 ± 0.65[a] | 60.38 ± 0.35[a] |
| $T_3$ (10g.kg) | 92.14 ± 1.43[a] | 1.87 ± 0.03[b] | 6.45 ± 0.10[a] | 66.84 ± 0.08[a] | 61.59 ± 1.03[a] |
| **Week 47** | | | | | |
| $T_1$ (Control) | 80.00 ± 0.71[b] | 2.34 ± 0.03[a] | 5.60 ± 0.05[b] | 61.37 ± 0.18[b] | 49.10 ± 0.58[b] |
| $T_2$ (5g.kg) | 92.85 ± 0.71[a] | 1.82 ± 0.02[b] | 6.50 ± 0.02[a] | 68.22 ± 0.37[a] | 63.35 ± 0.14[a] |
| $T_3$ (10g.kg) | 91.42 ± 0.71[a] | 1.86 ± 0.02[b] | 6.40 ± 0.05[a] | 67.81 ± 0.43[a] | 61.99 ± 0.08[a] |
| **Week 40-47** | | | | | |
| $T_1$ (Control) | 86.20 ± 1.38[b] | 2.25 ± 0.04[a] | 48.27 ± 0.77[b] | 59.39 ± 0.06[b] | 51.19 ± 0.88[b] |
| $T_2$ (5g.kg) | 92.86 ± 1.07[a] | 1.90 ± 0.01[b] | 52.00 ± 0.05[a] | 65.04 ± 0.29[a] | 60.39 ± 0.42[a] |
| $T_3$ (10g.kg) | 92.90 ± 0.18[a] | 1.93 ± 0.05[b] | 52.05 ± 0.10[a] | 64.10 ± 0.09[a] | 59.55 ± 0.03[a] |

Means with different letters in the same column indicate a significant difference level ($p<0.05$).

**Table 3:** Influence of thyme powder (*Thymus vulgaris*) on egg quality of hens (56 days).

| Parameters | Treatments | | |
|---|---|---|---|
| | $T_1$ (Control) | $T_2$ (5 g/Kg) | $T_3$ (10 g/Kg) |
| Haugh unit | 82.09 ± 3.04[a] | 86.18 ± 1.17[a] | 85.87 ± 1.12[a] |
| Yolk weight (g) | 28.03 ± 1.18[a] | 26.59 ± 0.75[a] | 28.32 ± 2.01[a] |
| Albumin weight (g) | 59.73 ± 0.98[a] | 59.02 ± 1.06[a] | 58.83 ± 1.85[a] |
| Eggshell weight (g) | 12.24 ± 0.82[a] | 12.39 ± 0.42[a] | 12.85 ± 0.48[a] |
| Albumin index (%) | 0.084 ± 0.006[b] | 0.101 ± 0.005[a] | 0.097 ± 0.001[ab] |
| Yolk index (%) | 0.449 ± 0.009[a] | 0.483 ± 0.022[a] | 0.446 ± 0.021[a] |
| Eggshell index (%) | 78.85 ± 0.87[b] | 82.01 ± 0.61[a] | 77.19 ± 0.41[ab] |
| Eggshell with member | 0.345 ± 0.008[a] | 0.390 ± 0.004[a] | 0.360 ± 0.006[a] |

Means with different letters in the same column indicate a significant difference level ($p<0.05$).

**Table 4:** Influence of thyme powder (*Thymus vulgaris*) on PCV (%), RBC ($10^6$ cells/mm$^3$), and WBC ($10^6$ cells/mm$^3$) of hens (56 days).

| Parameters | Treatments | | |
|---|---|---|---|
| | $T_1$ (Control) | $T_2$ (5 g/kg) | $T_3$ (10 g/kg) |
| PCV (%) | 30.33 ± 1.58$^b$ | 30.33 ± 2.57$^b$ | 32.16 ± 2.57$^a$ |
| RBC ($10^6$ cells/mm$^3$) | 2.64 ± 0.20$^a$ | 2.50 ± 0.06$^a$ | 2.57 ± 0.12$^a$ |
| WBC ($10^6$ cells/mm$^3$) | 22.77 ± 1.22$^b$ | 25.41 ± 0.79$^a$ | 25.41 ± 1.39$^a$ |

Means with different letters in the same column indicate a significant difference level (p<0.05).

**Table 5:** Effect of thyme powder (*Thymus vulgaris*) on lipid profiles and liver function of hens (56 days).

| Parameters | Treatments | | |
|---|---|---|---|
| | $T_1$ (Control) | $T_2$ (5 g.kg) | $T_3$ (10 g.kg) |
| Cholesterol (mg/dL) | 150.33 ± 2.12$^a$ | 147.00 ± 1.48$^b$ | 130.35 ± 1.34$^b$ |
| Triglycerides (mg/dL) | 169.83 ± 6.55$^a$ | 166.5 ± 6.50$^a$ | 164.00 ± 6.39$^a$ |
| ALT (I/U) | 41.66 ± 0.28$^a$ | 40.50 ± 0.49$^b$ | 40.83 ± 0.46$^b$ |
| AST (I/U) | 91.00 ± 1.22$^a$ | 73.83 ± 1.47$^b$ | 80.16 ± 1.35$^b$ |

Means with different letters in the same column indicate a significant difference level (p<0.05).

Recently, several studies have discovered that aromatic plants have a beneficial effect on animal production and physiology. Specifically, there is an increase in digestive enzyme secretion and improvements in immunity response and the gut flora population. This study evaluated the possible effects of supplementing laying hens with thyme powder in their diet on egg production, hematological, biochemical and some antioxidant parameters. Thyme treatments led to a significant improvement in the productive parameters in terms of an increase in egg number and mass and also improved feed conversion ratio when compared with the control. Some previous studies have shown that thyme powder or extracts improve egg production parameters (Abd El-Hack and Alagawany, 2015; Abdel-Wareth, 2016). Aydogan et al. (2020) showed that essential oils produced from medicinal plants can be successfully used as growth promoters in laying hens because their aromatic properties increase feed intake, and that herbs, such as thyme, black cumin seeds, green tea, garlic, neem. Their essential oils, can potentially be used as an alternative to antibiotics and growth promoters. In this study, the significant effect on egg production (%), egg mass, and feed conversion could be attributed to the improvement in digestion and nutrient absorption and the overall health of the digestive system (Shahryar et al., 2011). The active ingredients in thyme enhance the digestive enzymes, such as amylase, protease, and lipase, resulting in better nutrient digestibility and enhanced egg production. In contrast, Ding et al. (2017) did not find any significant difference in egg production or egg mass when hens were fed thyme. In this study, a significant increase in the packed cell volume (PCV %), and the white blood cells (WBC) count when using thyme treatment. Serum cholesterol levels were also lower in the thyme-treated hens when compared with the control hens. Serum cholesterol levels were consistent with those of Mansoub (2015). Hashemipour et al. (2013) found thyme to have a significant positive impact on the lipid profile, and our findings also show that thyme enhances the serum lipid parameters and it is agreed with (Abd El-Hack and Alagawany, 2015).

Thyme additives in the diet of laying hens also significantly ($p$ < 0.05) increases glutathione peroxidase activity, while decrease malondialdehyde concentration, and ALT and AST activity. Ashour et al. (2014) found that consuming herbs or their extracts leads to an increase in some antioxidant enzymes, such as glutathione peroxidase (GSH), as well as a decrease in the malondialdehyde (MDA) level. As a result, increased levels of glutathione peroxidase may help maintain the antioxidant system in a constant state in laying hens and the biological effects of antioxidant activity should benefit the health of chickens. The presence of phenolic hydroxy treatments, which act as hydrogen donors to the proxy radicals produced in the first stage of lipid oxidation, is believed to be responsible for thyme's high biological activity as a natural antioxidant (Hashemipour et al., 2013). Paraskevopoulou et al. (1997) found that adding thyme in hens' diet prevents lipid oxidation in eggs kept in the refrigerator. Based on this study findings, it can be concluded that using thyme as a phytogenic feed additive can improve the nutritional and economic aspects of poultry breeding in the future. The current study results are in agreement with (Gumus et al., 2017), who found that essential oils in thyme enhances glutathione peroxidase activity and decreases the malondialdehyde concentration in the liver and serum.

## CONCLUSIONS AND RECOMMENDATIONS

Thyme addition to the diet of laying hens improved egg production, egg quality, and feed conversion ratio, along with increasing glutathione peroxidase activity, decreasing cholesterol level, malondialdehyde and ALT and AST activity. Therefore, the addition of 5 and 10 g/kg of thyme is recommended as beneficial supplementation levels for laying hen feed.

## ACKNOWLEDGMENTS

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## NOVELTY STATEMENT

This study is to evaluate thyme as an additive because it contains bioactive components such as thymol, carvacrol, linalool, a-terpineol, and 1,8-cineole. Thyme powder is expected to increase egg production, egg quality, and feed conversion ratio in laying hens, while also increasing glutathione peroxidase activity, decreasing cholesterol, and increasing antioxidant responses.

## AUTHOR'S CONTRIBUTION

AM was coordinator of the research and analyzed and interpreted the data. AM, AA and SR in the study were supervisor of data collection and wrote draft manuscripts. A and S was assistants of the collection of data.

### Conflicts of interest

The authors have declared no conflict of interest.

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