Effects of adding essential oils of rosemary, dill and chicory extract to diets on performance, egg quality and some blood parameters of laying hens subjected to heat stress

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**ABSTRACT**

To determine the effects of adding essential oils of rosemary, dill and chicory extract to diets on performance, egg quality and enzyme activity of laying hens subjected to heat stress, a total of 240 laying hens were used and based on a 2 × 2 × 2 factorial, eight diets, with five replicates, including basal diet with two levels of rosemary essential oil (0, 20 ml/100 kg), two levels of dill essential oil (0, 15 ml/100 kg) and two levels of chicory extract (0, 250 ml/100 kg) were fed to the experimental birds. Dietary dill had a significant effect on egg index under heat stress condition. The birds given rosemary, dill and chicory alone exhibited lower serum cholesterol and triglycerides concentration under thermoneutral and heat stress condition compared to those fed the basal diet (P < 0.05). Synergistic effects between dietary rosemary, dill and chicory significantly increased plasma glutathione peroxidase activity compared to those that received only rosemary, dill or chicory, which has been never reported (P < 0.05). In conclusion, the addition of single or combination of rosemary, dill and chicory can improve at least some production performance and antioxidant enzyme activity (glutathione peroxidase) in heat-stressed laying hens.

**Introduction**

High environmental temperature may negatively affect feed intake (FI), nutrient utilization, growth rate, egg production and quality, feed efficiency and immunity; moreover, heat stress results in more incidence rate of oxidative stress in heat-stressed birds (Khan, Naz, Nikousefat, Selvaggi, et al. 2012; Khan et al. 2014). Heat stress may also increase the mortality rate which leads to economic losses (Khan et al. 2011). Feed consumption during heat stress is suppressed which leads to reduced nutrient intake (Khan et al. 2014). Chand et al. (2016) reported that lipid peroxidation due to the destructive effects of increased reactive oxygen species on the cellular protein is increased under heat stress condition. The positive effect of thyme oil administration to birds on oxidant degradation prevention in poultry products (meat and egg) is reported (Khan, Naz, Nikousefat, Tufarelli, et al. 2012). It seems that using antioxidants may help laying hens to resist against free radicals produced under heat stress conditions (Gous and Morris 2005).

Antimicrobial resistance and antibiotic (as growth promoters) residuals in animal products have been criticized and considered as a global health problem (Khan, Naz, Javdani, et al. 2012; Khan, Nikousefat, Tufarelli, et al. 2012). A wide variety of natural and herbal feed additives have evaluated and introduced as suitable and safe alternatives for antibiotic growth promoters (Khan, Naz, Nikousefat, Tufarelli, et al. 2012; Khan Naz, Tufarelli, et al. 2012). Rosemary (Rosmarinus officinalis L.), herb of the Labiatae family, has been recognized as the plant with the highest anti-oxidative activity (Estevez et al. 2007). Rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid and the antioxidants carnosic acid and carnosol are the most important organic chemicals, which have been already extracted from rosemary (Nakatani 2000; Crowley 2008). The activity of carnosic acid, the extracted antioxidant from rosemary (Richheimer et al. 1996), is higher than carnosol (almost three times) and butylated hydroxytoluene and butylated hydroxyanisol (seven times) (Richheimer et al. 1996).

Chicory (Cicorium intybus L.), a perennial herb used as a palatable forage crop for ruminants (Li and Kemp 2005), and its root contain high levels of fructooligosaccharides and inulin. Inulin, a water-soluble polymer of fructose with β-(2-1) glycosidic bond, has been used to improve the gut microflora and its integrity. It has also been identified to promote gastrointestinal mineral absorption and modulate lipid metabolism with hypolipidaemic effects (Azorin-Ortuno et al. 2009). Inulin is also known as prebiotic because it selectively stimulates the growth of beneficial Lactobacillus and Bifidobacterium (Rehman et al. 2008). Dill, Anethum graveolens L., belongs to Apiaceae family and is the only type of Anethum. Dill essence includes limonene and carron and is the same as Carum carvi L. ones (Kabeczka 2002). Dill is widely used as a traditional medicinal plant to treat gastrointestinal disorders (Hosseinzadeh et al. 2002);
decrease blood total cholesterol, LDL-C, triglyceride (Yazdanparast and Alavi 2001; Yazdanparast and Bahramikia 2008) and glucose (Panda 2008), and increase HDL-C (Yazdanparast and Alavi 2001). It is also used as an antioxidant agent (Satyanarayana et al. 2004).

The current study was conducted to evaluate the individual effects and probable synergistic interaction between the two plant essential oils, rosemary and dill, and extract of chicory on performance, egg quality and blood parameters of laying hens reared under thermo-natural and heat stress conditions.

Materials and methods

Laboratory animals, experimental design and treatments

All experimental protocols adhered and were approved by the guidelines of the Animal Ethics Committee of Razi University (Kermanshah, Iran). Two hundred and forty Lohmann LSL-Lite laying hens (30 weeks of age) were weighed individually (1410 ± 140 g) and randomly assigned to eight treatments with five replicates and six birds in each replicate in a completely randomized design. An empty cage was kept between blocks to eliminate cross-feeding. The birds were placed in the cages and kept under 16 h of light/8 h dark cycle for 16 weeks. The birds were reared under normal temperature (18–20°C) for 11 weeks; then the temperature was increased gradually up to 31°C for 5 weeks. The birds were exposed to 12 h of 18–22°C, 3 h of 20–31°C, 5 h of 31°C and 4 h of 31–20°C. Average ambient relative humidity inside the rearing house was 40%. The feed was offered on the basis of the recommendations (110 g/hen/day), and water (18 ± 4°C) was supplied ad libitum. According to the data given in Lohmann LSL-Lite catalog requirements (Lohmann LSL-Classic International 2011), a corn-soybean meal basal diet, 16.82% crude protein and 2720 kilocalories of metabolizable energy per kg of feed was formulated, since we tried to maintain constant ME:CP ratio, the percentage of crude protein was calculated as 16.82% (Table 1). Supplemental rosemary (20 ml/100 kg diet), dill (15 ml/100 kg diet) essential oil and chicory extract (250 g/100 kg) were purchased from Barij Essence Pharmaceutical Co, Kashan, Iran, according to the information received from the manufacturer, essential oil of rosemary contained 17.05% cineole, 26% α-pinene, 3.86% limonene, 3.98% borneol and 1.81% p-cymene, essential oil of dill contained 30.12% carvone and chicory extract contained 79 mg/ml inolin. The birds received one of the eight experimental diets including: (1) the basal diet (BD) (control group), (2) BD supplemented with chicory, (3) BD supplemented with dill, (4) BD supplemented with chicory+dill, (5) BD supplemented with rosemary, (6) BD supplemented with rosemary+chicory, (7) BD supplemented with rosemary+dill and (8) BD supplemented with rosemary+dill+chicory.

Productive performance and egg quality

The productive performance of the laying hens including hensday egg production (HDEP), FI and egg weight (EW) were recorded daily, and feed conversion ratio (FCR) and egg mass were calculated. To evaluate egg quality traits including yolk colour, eggshell thickness, eggshell weight, egg index, egg yolk index, yolk weight, albumen weight and Haugh unit, random samples of eggs (n=30) from each treatment, prior to the heat stress (week 41) and during the heat stress (week 46), were collected. Albumen height was determined by using a micrometer. Haugh units were calculated from the records of albumen height and EW using the Haugh unit formula (Eisen et al. 1962). The shell thickness was a mean value of measurement at three locations on the egg (air cell, equator and sharp end) by using a dial and pipe gage. Yolk colour was measured using the DSM colour fan. Specific gravity of eggs was determined by using the saline flotation method of Hempe et al. (1988). Common salt (NaCl) solutions were made in incremental concentrations of 0.005 in the range from 0.1065 to 0.1120 g/L. Yolk height (H) and yolk diameter (D) were measured by a tripod micrometer (Mitutoyo, 0.01 mm, Japan) and compass (Swordfish, 0.02 mm, China), respectively, then the yolk index was calculated. Eggs were broken and yolk and albumen were weighed separately. Egg length and width were individually recorded and used to calculate egg shape index.

Blood biochemical parameters

On weeks 41 (prior to the heat stress) and 46 (during the heat stress) of age, blood samples (3.0 ml) were collected from the bronchial wing vein of six hens from each treatment (one hen per each replicate cage) into tubes with or without anticoagulant (heparin). The collected blood samples were centrifuged at 3000 rpm (1008g) for 10 min, and the sera and plasma were frozen at −20°C until the analysis. Serum samples were thawed at room temperature and were analysed for glucose, triglyceride and cholesterol (Paris Azmun, Tehran, Iran); besides, the enzyme activity of plasma glutathione peroxidase was measured (Randox Laboratories kit, Antrim, UK).

Table 1. Composition of the basal diet.

| Ingredient          | Percentage |
|---------------------|------------|
| Corn                | 62.14      |
| Soybean meal        | 25.95      |
| Soybean oil         | 0.69       |
| Dicalcium phosphate | 1.39       |
| Oyster shell        | 8.78       |
| Common salt         | 0.34       |
| Vitamin premix a    | 0.25       |
| Mineral premix b    | 0.25       |
| Methionine          | 0.21       |

aVitamin mixture per 2.5 kg of diet provides the following: vitamin A, 7,700,000 IU; vitamin D3, 3,300,000 IU; vitamin E, 6600 mg; vitamin K3, 550 mg; thiamine, 2200 mg; riboflavin, 4400 mg; vitamin B6, 4400 mg; capantothenate, 550 mg; nicotinic acid, 200 mg; folic acid, 110 mg; choline chloride, 275,000 mg; biotin, 55 mg; vitamin B12, 8.8 mg.

bMineral mixture per 2.5 kg of diet provides the following: Mn, 66,000 mg; Zn, 66,000 mg; Fe, 33,000 mg; Cu, 8800 mg; Se, 300 mg; I, 900 mg.
Statistical analysis

The data were subjected to ANOVA in a completely randomized design with a $2 \times 2 \times 2$ factorial arrangement of treatments using GLM procedure of SAS (SAS, 2003). All statements of significance are based on a probability of <.05. The mean values were compared by Duncan's multiple-range test. The following model was considered for analysis:

$$Y_{ijk} = \mu + (R_i) + (C_j) + (D_k) + (RC_{ij}) + (RD_{ik}) + (CD_{jk}) + (RCD_{ijk}) + (e_{ijkl})$$

where $Y_{ijk}$ is the measured characteristic, $\mu$ is the overall mean, $(R_i)$ is the main effect of rosemary, $(C_j)$ is the main effect of chicory, $(D_k)$ is the main effect of dill, and $(RC_{ij})$, $(RD_{ik})$, $(CD_{jk})$, $(RCD_{ijk})$ are interaction between the effect of two or three factors and $(e_{ijkl})$ is the residual error. The effects of the main factors were not considered, whenever the interaction was significant.

Results

Productive performance

The effects of dietary supplemental chicory, rosemary and dill on the performance of laying hens are shown in Tables 2 and 3. In general, no differences in FCR, HDEP and EW were observed in laying hens fed the experimental diets under normal temperature (30–41 weeks), heat stress condition (41–46 weeks) and

### Table 2. Effects of diet supplementation by rosemary (ml/100 kg), dill (ml/100 kg) and chicory (g/100 kg) on feed conversion ratio (g egg/g feed) and egg production (%) of laying hens reared under thermoneutral (Weeks 30–41 of age) and heat stress conditions (Weeks 41–46 of age).

| Treatments | Feed conversion ratio (g egg/g feed)* | Hen-day egg production (HDEP) (%) |
|------------|-------------------------------------|---------------------------------|
|            | Weeks 30–41 Before stress | Weeks 30–41 During stress | Weeks 30–41 Total period | Weeks 41–46 Before stress | Weeks 41–46 During stress | Weeks 41–46 Total period |
| Rosemary   |                              |                                 |                           |                          |                                 |                           |
| 0          | 1.90 ± 0.04                  | 1.93 ± 0.07                     | 1.91 ± 0.05               | 97.83 ± 0.72             | 96.66 ± 1.80                  | 97.43 ± 1.15               |
| 20         | 1.90 ± 0.05                  | 1.91 ± 1.00                     | 1.91 ± 0.06               | 97.91 ± 0.87             | 96.67 ± 2.08                  | 97.02 ± 1.80               |
| Dill       |                              |                                 |                           |                          |                                 |                           |
| 0          | 1.89 ± 0.04                  | 1.92 ± 0.09                     | 1.91 ± 0.06               | 97.93 ± 0.72             | 97.02 ± 1.60                  | 97.20 ± 1.25               |
| 15         | 1.91 ± 0.04                  | 1.92 ± 0.07                     | 1.91 ± 0.05               | 97.91 ± 0.67             | 97.57 ± 1.86                  | 97.30 ± 1.54               |
| Chicory    |                              |                                 |                           |                          |                                 |                           |
| 0          | 1.91 ± 0.04                  | 1.93 ± 0.09                     | 1.92 ± 0.05               | 97.93 ± 0.72             | 97.02 ± 1.60                  | 97.20 ± 1.25               |
| 250        | 1.89 ± 0.04                  | 1.92 ± 0.09                     | 1.92 ± 0.07               | 97.81 ± 0.79             | 97.24 ± 2.34                  | 97.12 ± 2.01               |
| SEM**      | 0.0063                      | 0.0121                          | 0.0088                    | 0.112                    | 0.304                         | 0.250                      |
| CV         | 2.28                        | 4.42                            | 3.21                      | 0.77                     | 2.04                          | 1.79                       |
| P value    |                              |                                 |                           |                          |                                 |                           |
| R          | 0.79                        | 0.36                            | 0.90                      | 0.75                     | 0.15                          | 0.33                       |
| D          | 0.29                        | 0.74                            | 0.90                      | 0.40                     | 0.16                          | 0.64                       |
| CH         | 0.09                        | 0.58                            | 0.49                      | 0.61                     | 0.83                          | 0.90                       |
| R/D        | 0.67                        | 0.38                            | 0.86                      | 0.47                     | 0.56                          | 0.69                       |
| D/CH       | 0.94                        | 0.22                            | 0.59                      | 0.29                     | 0.38                          | 0.85                       |
| R/D/CH     | 0.25                        | 0.88                            | 0.78                      | 0.85                     | 0.20                          | 0.60                       |
|            |                              |                                 |                           |                          |                                 |                           |

Note: Values within a column with different superscripts differ significantly at $P \leq 0.05$.

*Values are expressed as mean ± SD (standard deviation).

**Standard error of the mean.

### Table 3. Effects of diet supplementation by rosemary (ml/100kg), dill (ml/100kg) and chicory (g/100kg) on egg mass (g egg/g feed) and egg weight (g) of laying hens reared under thermoneutral (Weeks 30–41 of age) and heat stress conditions (Weeks 41–46 of age).

| Treatments | Egg mass (g/hen/day)a | Egg weight (g) |
|------------|------------------------|---------------|
|            | Weeks 30–41 Before stress | Weeks 30–41 During stress | Weeks 30–41 Total period | Weeks 41–46 Before stress | Weeks 41–46 During stress | Weeks 41–46 Total period |
| Rosemary   |                              |                                 |                           |                          |                                 |                           |
| 0          | 57.5 ± 1.45              | 57.0 ± 1.98                      | 57.3 ± 1.44               | 58.9 ± 1.42             | 58.8 ± 1.98                  | 58.9 ± 1.52               |
| 20         | 57.7 ± 1.53              | 57.1 ± 2.04                      | 57.5 ± 1.95               | 59.0 ± 1.28             | 59.1 ± 1.42                  | 59.0 ± 1.26               |
| Dill       |                              |                                 |                           |                          |                                 |                           |
| 0          | 57.8 ± 1.64              | 57.5 ± 2.19                      | 57.8 ± 1.87               | 59.1 ± 1.55             | 59.5 ± 1.74                  | 59.2 ± 1.56               |
| 15         | 57.4 ± 1.31              | 56.6 ± 1.69                      | 57.3 ± 1.56               | 58.7 ± 1.08             | 58.5 ± 1.56                  | 58.6 ± 1.15               |
| Chicory    |                              |                                 |                           |                          |                                 |                           |
| 0          | 57.3 ± 1.22              | 56.3 ± 1.49                      | 57.2 ± 1.55               | 58.6 ± 1.02             | 58.5 ± 1.64                  | 58.6 ± 1.09               |
| 250        | 57.8 ± 1.68              | 57.7 ± 2.19                      | 57.6 ± 1.85               | 59.3 ± 1.55             | 59.4 ± 1.69                  | 59.3 ± 1.56               |
| SEMb       | 0.225                    | 0.277                            | 0.263                     | 0.195                   | 0.234                       | 0.200                     |
| CV         | 2.74                     | 3.33                             | 3.21                      | 2.32                    | 2.78                        | 2.37                      |
| P value    |                              |                                 |                           |                          |                                 |                           |
| R          | 0.74                     | 0.79                             | 0.84                      | 0.79                    | 0.58                        | 0.72                      |
| D          | 0.50                     | 0.12                             | 0.80                      | 0.32                    | 0.06                        | 0.19                      |
| CH         | 0.34                     | 0.02                             | 0.50                      | 0.13                    | 0.08                        | 0.10                      |
| R/D        | 0.98                     | 0.23                             | 0.81                      | 0.52                    | 0.59                        | 0.59                      |
| R/CH       | 0.72                     | 0.65                             | 0.59                      | 0.59                    | 0.34                        | 0.94                      |
| D/CH       | 0.63                     | 0.44                             | 0.66                      | 0.94                    | 0.22                        | 0.64                      |
| R/D/CH     | 0.58                     | 0.72                             | 0.72                      | 0.28                    | 0.64                        | 0.36                      |

Note: Values within a column with different superscripts differ significantly at $P \leq 0.05$.

aValues are expressed as mean ± SD (standard deviation).

bStandard error of the mean.
overall period of the experiment (30–46 weeks) \((P > 0.05)\). There was a significant effect of dietary supplemental chicory on egg mass during heat stress (41–46 weeks). The highest egg mass was observed in the hens fed 250 ml/100 kg supplemental chicory compared to the control group.

### Egg quality

The effects of dietary supplemental chicory, rosemary and dill on the egg quality traits at different periods of the experiment are shown in Tables 4–10. Since the significant interaction effects between chicory and/or rosemary and/or dill on eggshell weight, Haugh unit, egg index, yolk index and glutathione enzyme activity were observed, the main effects of them (chicory, rosemary and dill) are not shown in Tables 7–10. No significant effects of dietary treatments were detected on egg gravity, yolk weight, albumen weight and yolk colour \((P > 0.05)\). Significant interactions between dietary chicory and dill on egg index and eggshell weight prior to the heat stress and on Haugh unit during the heat stress were observed \((P < 0.05)\), so that the laying hens fed the diet added 250 ml/100 kg chicory had the highest Haugh unit (73.88) compared to the control group.

#### Table 4. Effects of diet supplementation by rosemary (ml/100 kg), Dill (ml/100 kg) and chicory (g/100 kg) on gravity, egg colour and yolk weight (g) of laying hens reared under thermoneutral (Week 41 of age) and heat stress conditions (Week 46 of age).

| Age of sampling Heat stress | Week 41 | Week 46 |
|---------------------------|--------|--------|
| Before heat stress        |        |        |
| During heat stress        |        |        |
| **Egg gravity**           |        |        |
| Treatments                |        |        |
| Rosemary                  |        |        |
| 0                         | 4.10 ± 0.31 | 4.20 ± 0.41 |
| 20                        | 4.05 ± 0.22 | 4.25 ± 0.44 |
| Dill                      |        |        |
| 0                         | 4.10 ± 0.31 | 4.15 ± 0.37 |
| 15                        | 4.05 ± 0.22 | 4.30 ± 0.47 |
| Chicory                   |        |        |
| 0                         | 4.10 ± 0.31 | 4.10 ± 0.31 |
| 250                       | 4.05 ± 0.22 | 4.35 ± 0.49 |
| SEMb                      | 0.039  | 0.062  |
| CV                        | 6.72   | 10.24  |

*Note: Values within a column with different superscripts differ significantly at \(P \leq 0.05\).*

**Values are expressed as mean ± SD (standard deviation).**

**Standard error of the mean.**

#### Table 5. Effects of diet supplementation by rosemary (ml/100 kg), Dill (ml/100 kg) and chicory (g/100 kg) on Haugh unit, shell weight (g) and shell thickness \((\times 10^{-2}\text{ mm})\) of laying hens reared under thermoneutral (Week 41 of age) and heat stress conditions (Week 46 of age).

| Age of sampling Heat stress | Week 41 | Week 46 |
|---------------------------|--------|--------|
| Before heat stress        |        |        |
| During heat stress        |        |        |
| **Haugh unit**            |        |        |
| Treatments                |        |        |
| Rosemary                  |        |        |
| 0                         | 69.3 ± 2.76 | 70.9 ± 3.11 |
| 20                        | 70.7 ± 2.74 | 72.1 ± 2.40 |
| Dill                      |        |        |
| 0                         | 69.9 ± 2.59 | 71.9 ± 2.95 |
| 15                        | 70.1 ± 3.09 | 71.1 ± 2.66 |
| Chicory                   |        |        |
| 0                         | 69.7 ± 3.21 | 70.6 ± 2.66 |
| 250                       | 70.3 ± 2.39 | 72.4 ± 2.72 |
| SEMb                      | 0.395  | 0.330  |
| CV                        | 3.95   | 3.23   |

*Note: Values within a column with different superscripts differ significantly at \(P \leq 0.05\).*

**Values are expressed as mean ± SD (standard deviation).**

**Standard error of the mean.**

**Standard error of the mean.**
Table 6. Effects of diet supplementation by rosemary (ml/100kg), Dill (ml /100kg) and chicory (g/100kg) on egg index (%),yolk index (%) and albumen weight (g) of laying hens reared under thermoneutral (Week 41 of age) and heat stress conditions (Week 46 of age).

| Age of sampling Heat stress | Egg index (%) | Yolk index (%) | Albumen weight (g) |
|-----------------------------|---------------|----------------|-------------------|
|                             | Before heat stress | During heat stress | Before heat stress | During heat stress | Before heat stress | During heat stress |
| Treatments                  | Week 41        | Week 46         | Week 41           | Week 46           | Week 41           | Week 46           |
| Rosemary                    | 0              | 20              | 15:250            | 20:15:250         | 20:0:250          | 0:250             |
|                            | 41.7 ± 1.48    | 38.9 ± 1.41     | 34.9 ± 1.68       | 33.8 ± 2.14       |
|                            | 41.6 ± 1.46    | 38.9 ± 1.42     | 35.3 ± 1.74       | 34.6 ± 1.71       |
|                            | 41.9 ± 1.57    | 38.9 ± 1.66     | 35.1 ± 1.83       | 34.1 ± 1.82       |
|                            | 41.9 ± 1.32    | 38.9 ± 1.16     | 35.1 ± 1.61       | 34.3 ± 2.10       |
|                            | 41.1 ± 0.59    | 38.9 ± 1.16     | 35.1 ± 1.61       | 34.3 ± 2.10       |
| Dill                       | 0              | 20              | 15:250            | 20:15:250         | 20:0:250          | 0:250             |
|                            | 41.9 ± 1.69    | 38.4 ± 1.20b    | 35.6 ± 1.33       | 34.3 ± 2.09       |
|                            | 41.4 ± 1.15    | 39.4 ± 1.43b    | 34.7 ± 1.94       | 34.1 ± 1.85       |
|                            | 40.1 ± 0.77    | 38.9 ± 1.16     | 35.1 ± 1.83       | 34.1 ± 1.50       |
|                            | 41.9 ± 0.59    | 38.9 ± 1.16     | 35.1 ± 1.61       | 34.3 ± 2.10       |
|                            | 41.9 ± 0.59    | 38.9 ± 1.16     | 35.1 ± 1.61       | 34.3 ± 2.10       |
| Chicory                    | 0              | 20              | 15:250            | 20:15:250         | 20:0:250          | 0:250             |
|                            | 42.9 ± 0.92    | 38.9 ± 1.16     | 35.1 ± 1.83       | 34.1 ± 1.82       |
|                            | 42.6 ± 0.92    | 38.9 ± 1.16     | 35.1 ± 1.61       | 34.3 ± 2.10       |
|                            | 41.9 ± 0.92    | 38.9 ± 1.16     | 35.1 ± 1.61       | 34.3 ± 2.10       |

Note: Values within a column with different superscripts differ significantly at P ≤ 0.05.
*Values are expressed as mean ± SD (standard deviation).

Table 7. Eggshell weight (g) before heat stress condition when interactions between Dill+Chicory were significant.

| Treatments | Eggshell weight (g) |
|------------|---------------------|
| Dill/Chicory (ml/100 kg) | |
| 0          | 74.4 ± 0.97         |
| 20         | 74.4 ± 1.51         |
| 15         | 74.1 ± 1.06         |
| 250        | 74.1 ± 1.06         |
| SEM         | 0.162               |
| CV          | 1.51                |

Table 8. Eggshell weight (g) under heat stress condition when interactions between Rosemary+Chicory and Rosemary+Dill were significant.

| Treatments | Eggshell weight (g) |
|------------|---------------------|
| Rosemary/Chicory (ml/100 kg) | Eggshell weight (g) |
| 0          | 5.73 ± 0.17         |
| 0.250      | 5.93 ± 0.09         |
| 15         | 5.94 ± 0.17         |
| 15:250     | 5.90 ± 0.14         |

Note: Values within a column with different superscripts differ significantly at P ≤ 0.05.
*Values are expressed as mean ± SD (standard deviation).

Table 9. Haugh unit (under heat stress condition) and egg index (before heat stress) when interactions between Dill+Chicory were significant.

| Treatments | Haugh unit (under heat stress condition) | Egg index (before heat stress) |
|------------|----------------------------------------|-------------------------------|
| 0          | 69.9 ± 1.81c                        | 73.8 ± 0.93c                 |
| 0.250      | 73.9 ± 2.52a                        | 74.1 ± 1.39b                 |
| 15         | 71.3 ± 3.25b                        | 76.0 ± 1.30ab                |
| 15:250     | 70.9 ± 2.07c                        | 74.1 ± 0.80b                 |

Note: Values within a column with different superscripts differ significantly at P ≤ 0.05.
*Values are expressed as mean ± SD (standard deviation).

Table 10. Yolk index (before heat stress) and glutathione enzyme activity (under heat stress) when interactions between rosemary, chicory and Dill were significant.

| Treatments | Yolk index (before heat stress) | Glutathione enzyme activity (under heat stress) |
|------------|---------------------------------|-----------------------------------------------|
| 0.00       | 42.5 ± 1.840                   | 653.3 ± 19.63b                               |
| 0.250      | 42.1 ± 1.47ab                  | 1384.2 ± 242.03a                             |
| 0.150      | 40.2 ± 0.61bc                  | 1041.2 ± 510.16b                             |
| 0.15:250   | 41.9 ± 0.60abc                 | 879.5 ± 339.46b                              |
| 20.00      | 40.6 ± 1.27abc                 | 1186.0 ± 497.62ab                            |
| 0.20:250   | 42.5 ± 1.78ab                  | 859.0 ± 95.93ab                              |
| 0.15:00    | 42.4 ± 0.98abc                 | 1030.0 ± 109.40ab                            |
| 20:15:250  | 41.0 ± 0.97abc                 | 1104.2 ± 208.27ab                            |

Note: Values within a column with different superscripts differ significantly at P ≤ 0.05.
*Values are expressed as mean ± SD (standard deviation).
Blood parameters

The effects of dietary supplemental chicory, rosemary and dill on the serum concentrations of glucose, triglyceride and cholesterol of hens under thermoneutral or heat stress conditions are presented in Table 11. There was no significant difference between serum glucose concentrations in all the experimental groups. Dietary supplemental dill decreased serum cholesterol and triglyceride levels under the heat stress condition compared to the control group ($P < 0.05$). Serum triglyceride concentrations were not significantly affected by dietary supplemental rosemary ($P > 0.05$); however, rosemary decreased serum cholesterol under the heat stress condition compared to the control group ($P < 0.05$). The laying hens fed chicory-included diet showed lower serum triglyceride and cholesterol concentrations compared to the control group ($P < 0.05$). The effects of dietary supplemental chicory, rosemary and dill on the activity of plasma glutathione are also presented in Table 11. Three-way interactions between dietary treatments on glutathione activity were observed ($P < 0.05$), so that the treatments containing 250 ml/100 kg chicory and the control group had the highest and the lowest glutathione enzyme activity among dietary groups, respectively (Table 10).

Discussion

Productive performance

In the current study, diet supplementation with rosemary had no significant effect on production performance of the laying hens. There are contradictory reports about the effect of rosemary on poultry performance. Rahman et al. (2017) detected a negative effect of VitE (as an antioxidant) supplementation on FCR of broilers. Adding 0.5% or 1% thyme, oregano or rosemary leaves to laying hen diets improved FCR (Radwan et al., 2008). According to Yesilbag et al. (2013), egg production and feed efficiency were significantly improved in the quails fed the diets supplemented by rosemary essential oil compared to control; however, no significant effect of treatments was seen on EW and egg mass. Kassie (2008) confirmed that adding anise (0.5 g/kg) and rosemary essential oil (1 g/kg) significantly improved FCR in broilers. These results may be due to the positive effects of aromatic herbs and their essential oils on the digestion via improving the activity of hydrolysing enzymes (Jamroz and Kamel, 2002; Basmacioglu et al., 2004). Nevertheless, there are some studies showing negative effects of mixtures of active compounds and oils on the performance parameters (Lee et al. 2003; Botoglo et al. 2004). Environmental condition, management, nutrition, type and dosage of additive as well as birds’ related factors (age, species and production stage) may be partly responsible for these contradictory results. In the current study, no significant effects of chicory were seen on the FCR, egg production and EW, but improved egg mass was observed under heat stress condition in the laying hens fed chicory-included diet compared to those fed the control diet. Yildiz et al. (2006) reported no significant effect of dietary supplemental inulin in the form of dried Jerusalem artichoke, Helianthus tuberosus L. on laying rate, feed consumption and FCR. Radwan et al. (2008) observed better FCR in the 28-week-old local laying hens fed Vit E. Besides, Chen et al. (2005) recorded an improved FCR in 57-week-aged White Leghorn hens received diet supplemented with semipurified chicory inulin extract. Ayasan et al. (2005) showed that diet supplementation with Yucca schidigera tended to improve FCR in female Japanese quails. Świątkiewicz et al. (2010) reported no significant effects of dietary prebiotics on egg performance, egg mass, FI and FCR in laying hens. In contrast, Park and Park (2012) reported that dietary supplemental inulin (250 mg/kg diet) increased egg production and weight in laying hens. Chen et al. (2005) observed that the inclusion of 1% inulin or oligofructose in the laying hens’ diet improved egg production. Various reactions of the birds to diet supplementation by prebiotics may be partly related the composition of the basal diet (i.e. the content of non-digestible oligosaccharides), dietary concentration of the additives, and the farm environmental and hygiene conditions (Verdonk et al. 2005).

Stressors have negative effects on the gut microflora balance (Lan et al. 2004). The positive effects of prebiotics in heat-stressed broilers have been shown by Sohail et al. (2010). The results of the current study showed no significant effect of dietary dill on egg production, egg mass, FCR and EW. Diet supplementation with prebiotics can help birds to overcome deficiency and dominantly increase their tolerance to stress; in the same report, it was suggested that the positive effect of chicory supplementation on egg mass could be attributed to the effects of prebiotics on gut microflora (Ghareeb et al. 2008).

Egg quality

Shell thickness in the laying hens fed the chicory-included diet under thermoneutral conditions (30–41 weeks) was higher compared to the control group. In addition, there were significant interactions between chicory and dill on Haugh unit and eggshell weight under heat stress condition. Significant interactions between chicory and rosemary, rosemary and dill on eggshell weight were also observed under heat stress condition. The interaction between chicory and dill was significant on egg index prior to the heat stress condition. Dietary supplementation by dill had a significant effect on egg index under heat stress condition. Three-way interaction between dietary treatments on yolks index prior to the heat stress was observed. Haugh unit and eggshell thickness were higher when the inulin oligosaccharide added to diet (250 mg INO kg/diet), compared to the control group (Park and Park 2012), due to increased absorption rate of nutrients such as calcium, which is the main component of eggshell (Azorin-Ortuno et al. 2009). In addition, it is reported that inulin may improve mineral absorption rate through the synergistic action of multiple Bifidobacterium and short chain fatty acid in human colon (Mitsuoka, 1990). According to the report by Świątkiewicz et al. (2010), the prebiotic fructans had a considerable effect on eggshell quality at 46, 58 and 70 weeks of age in laying hens. The results obtained by Chen and Chen (2004) indicated that supplementation of 1% oligofructose or inulin to the diet, significantly increased eggshell weight and breaking strength. Yildiz et al. (2006) found no effect of inulin from dried Jerusalem artichoke, Helianthus tuberosus L. on laying rate, feed consumption and FCR.
artichoke on the weight, thickness and breaking strength of eggshell during a 16-week trial. Thus, it can be suggested that the improvement in egg shell quality in this study might be resulted from the increased mineral absorption.

There are a few studies evaluating usage of aromatic plants and their extracts on performance of laying hens. Botsoglou et al. (2005) reported no significant effects of adding rosemary, oregano, saffron and α-tocopheryl acetate to diet on EW, egg shape index, yolk shape index, Haugh unit and egg shell thickness. According to Radwan et al. (2008), there were no significant effects of rosemary on shell weight, shell thickness, egg shape index, yolk colour and Haugh unit in laying hens. Yesilbag et al. (2013) reported no significant effect of adding rosemary volatile oil (200 mg/kg) to diet of the quails on shape index, Haugh unit and egg shell thickness values, but egg albumen and yolk index were increased. Panagiota et al. (2006) reported no significant effect of adding rosemary and α-Tocopherol acetate to diet on EW and shape index, yolk diameter, height and colour, Haugh unit and shell thickness. Bozkurt et al. (2012) found that neither an antibiotic growth promoter nor an herbal essential oil mixture caused a significant effect on egg quality parameters with the exception of Haugh unit. Gharaghani et al. (2015) by adding 0, 10, 15 and 20 g of fennel (Foeniculum vulgare Mill.) per kg of diet showed the anti-oxidative properties of fennel fruit in alleviating the detrimental effects of free radicals produced in heat stress conditions.

**Blood parameters**

Dietary supplemental dill had no significant effect on serum glucose but decreased serum cholesterol and triglyceride in laying hens reared under heat stress. According to Yazdanparast and Bahramikia (2008), the water extract of dill leaf reduced the total triglyceride and total cholesterol in hyperlipidaemia male rats. Hosseini (2011) showed that adding oregano oil to diet had a significant effect on triglyceride, cholesterol, LDL-C and HDL-C levels. The proposed mechanism by which dill can reduce cholesterol is reducing lipid absorption in intestine by binding bile acids, which results in increasing lipid excretion and reducing blood lipid concentration. Qureshi et al. (1983) suggested the reduced activity of enzymes involved in lipid metabolism including 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (enzyme associated with cholesterol synthesis), cholesterol-7 hydroxylase fatty acid synthase and pentose phosphate pathway as the possible mechanisms in reducing lipid due to consuming of herbal products. Hajhashemi and Abbasi (2008) reported that adding dill essential oils to the diet of rats reduced the plasma cholesterol and triglyceride levels. Bano et al. (2013) indicated that oral administration of aqueous extract of *Anethum graveolens* seeds reduced blood total cholesterol and triglyceride in overweight rats. Bahadori et al. (2013) reported that dill powder had no significant effect on glucose, triglyceride and cholesterol levels in broilers. In the present study, adding essential oil of rosemary to diet decreased the serum concentration of triglyceride under thermoneutral and serum cholesterol in thermoneutral and heat stress conditions. Radwan et al. (2008) reported that adding thyme or rosemary to diet decreased total lipid, total cholesterol and LDL-C. Radwan (2003) observed the reducing effect of thymol and carvacrol on HMG-CoA, which in turn decreased total lipid and cholesterol. Lee et al. (2003) observed that dietary carvacrol significantly decreased plasma triglyceride and phospholipids, respectively. Bolukbasi et al. (2008) reported that adding essential oils of thyme, sage and rosemary reduced serum cholesterol and triglyceride in laying hens. In the present study, the significant effect of chicory on serum cholesterol and triglyceride was observed with no significant effect on serum glucose. Insulin is known to have a decreasing effect on the blood levels of lipid in human and animals (Fiordaliso et al., 1995). Based on the report by Causey et al. (2000),

### Table 11. Effects of diet supplementation by rosemary (ml/100kg), Dill (ml /100kg) and chicory (g/100kg) on glucose, triglyceride and cholesterol of laying hens reared under thermoneutral (Week 41 of age) and heat stress conditions (Week 46 of age)

| Treatments | Age of sampling | Glucose (mg/dl) | Triglyceride (mg/dl) | Cholesterol (mg/dl) |
|------------|----------------|----------------|---------------------|--------------------|
|            | Heat stress    | Week 41        | Week 46             | Week 41            | Week 46            | Week 41            | Week 46            |
|            | Before heat stress | During heat stress | Before heat stress | During heat stress | Before heat stress | During heat stress | Before heat stress | During heat stress |
| Rosemary   | 0              | 226.1 ± 19.63  | 238.0 ± 29.52       | 1192.6 ± 213.14    | 1436.7 ± 266.63    | 261.3 ± 60.32³    | 338.6 ± 59.05⁵    |
|            | 20             | 231.0 ± 20.08  | 237.8 ± 26.31       | 1081.4 ± 162.44    | 1327.8 ± 270.15    | 226.2 ± 50.70³    | 289.9 ± 74.96⁴    |
| Dill       | 0              | 233.3 ± 17.44  | 238.6 ± 22.50       | 1224.2 ± 193.46⁸   | 1470.3 ± 262.88⁸   | 273.1 ± 49.09⁹    | 343.1 ± 68.17⁷    |
|            | 15             | 223.5 ± 21.36  | 237.1 ± 32.51       | 1046.2 ± 156.71⁵   | 1294.1 ± 254.69⁵   | 214.4 ± 51.36⁶    | 285.4 ± 62.86⁶    |
| Chicory    | 0              | 231.0 ± 21.49  | 234.9 ± 30.49       | 1144.5 ± 209.20    | 1431.0 ± 282.71    | 250.5 ± 62.89      | 331.6 ± 77.06     |
|            | 250            | 226.3 ± 18.12  | 240.8 ± 24.82       | 1134.8 ± 187.29    | 1333.4 ± 255.69    | 237.0 ± 52.97      | 297.0 ± 61.52     |
| SEM        |                | 2.926          | 4.268               | 27.177             | 38.196             | 7.180             | 8.952             |
| CV         |                | 8.76           | 12.53               | 15.72              | 19.29              | 20.57             | 19.89             |
| P value    |                |               |                     |                    |                    |                  |                  |
| R          | 0.38           | 0.98          | 0.05                | 0.21               | 0.03              | 0.02             |
| D          | 0.12           | 0.87          | 0.004               | 0.04               | 0.0008           | 0.01             |
| CH         | 0.55           | 0.54          | 0.69                | 0.26               | 0.40             | 0.09             |
| R/D        | 0.41           | 0.60          | 0.46                | 0.93               | 0.61             | 0.65             |
| R/CH       | 0.72           | 0.77          | 0.87                | 0.92               | 0.79             | 0.81             |
| D/CH       | 0.48           | 0.87          | 0.94                | 0.87               | 0.66             | 0.71             |
| R/D/CH     | 0.26           | 0.43          | 0.98                | 0.40               | 0.96             | 0.61             |

Note: Values within a column with different superscripts differ significantly at P ≤ 0.05. *Values are expressed as mean ± SD (standard deviation). **Standard error of the mean.
adding chicory inulin to diet of humans with hypercholesterolaemia reduced blood triglyceride and total cholesterol. In addition, inulin reduced blood triglyceride mostly due to inhibiting gene expression of the lipogenic enzymes, which in turn decreased the synthesis of de novo fatty acid synthesis (Delzenne and Kok, 1999). Inulin reduced blood cholesterol via inhibiting cholesterol biosynthesis in the liver, and modulation of carbohydrate and lipid metabolism (Wolever et al., 1995). Moreover, it was reported that blood cholesterol was removed in part by fermentation products generated via the fermentation of carbohydrate and lipid metabolism (Wolever et al., 1995). Inulin reduced blood triglyceride mostly due to inhibiting gene expression of the lipogenic enzymes, which in turn decreased the synthesis of de novo fatty acid synthesis (Delzenne and Kok, 1999). Inulin reduced blood cholesterol via inhibiting cholesterol biosynthesis in the liver, and modulation of carbohydrate and lipid metabolism (Wolever et al., 1995). Moreover, it was reported that blood cholesterol was removed in part by fermentation products generated via the fermentation of carbohydrate and lipid metabolism (Wolever et al., 1995).

Another suggested way in which inulin can reduce blood cholesterol is inhibiting the synthesis of cholesterol in liver through both increasing excretion of bile acids and the inhibiting HMG-CoA reductase activity (Kim and Shin, 1998). Yusrizal and Chen (2003) observed that adding chicory-based fructans to diet decreased the serum cholesterol level in broilers. Safamehr et al. (2012) showed that adding 0.5% or 1% chicory to diet had no significant effect on blood glucose level of broiler. In the present experiment, three-way interaction between dietary treatments (rosemary, dill and chicory: 0, 0, 250 ml/100 kg, respectively) on glutathione enzyme activity under heat stress condition was observed. In general, there is not enough information regarding to the effect of adding chicory, rosemary or dill to diet on the enzyme activity of laying hens.

Conclusion
The results obtained in the current study show the positive effects of addition of single or combination of rosemary, dill and chicory can improve at least some egg quality and production performance parameters, namely, egg mass, eggshell weight, eggshell thickness, egg yolk index, egg index and egg mass, as well as antioxidant enzyme activity (glutathione peroxidase) in heat-stressed laying hens. In addition of these valuable positive effects, the more mentionable result of the current study could be the interaction effects of the rosemary, dill and/or chicory extracts on egg index, eggshell weight, Haugh unit and yolk index before and/or during heat stress condition.

Disclosure statement
No potential conflict of interest was reported by the authors.

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