Influence of dietary supplemental chromium and magnesium on performance and metabolic parameters of laying hens subjected to heat stress

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ABSTRACT
To determine the effects of adding supplemental Mg and Cr to diets on performance, egg quality, blood parameters and enzyme activity of laying hens subjected to heat stress, a total of 324 laying hens were used and based on a 3×3 factorial, nine diets, six replicates, including basal diet with Mg (0, 300 and 600 mg kg⁻¹ from Mg oxide) and three levels of Cr (0, 400, 800 μg kg⁻¹ from Cr methionine) were fed to the experimental birds. The temperature was increased gradually up to 31°C for 5 weeks. Feed conversion ratio and egg mass improved in the birds fed either 300 or 600 mg kg⁻¹ Mg compared to those fed the basal diet (P < 0.05). During the heat stress, birds fed the diets included 300 or 600 mg kg⁻¹ Mg produced eggs with higher shell thickness. Dietary supplemental Mg and Cr separately decreased blood uric acid in laying hens during heat stress (P < 0.05). It can be concluded that diet supplementation by Mg could have beneficial effects on feed conversion ratio, shell thickness and serum uric acid, whereas diet supplementation by Cr decreased serum glucose, total cholesterol, triglycerides and uric acid of laying hens under heat stress condition.

Introduction
Poultry producers commonly face the challenge of heat stress either seasonally or year-round. The detrimental effects of heat stress on laying hens range from reduced growth and egg production (EP) to decreased internal and external egg quality (Khan et al. 2014a). Feed consumption during heat stress is suppressed which leads to reduced nutrient intake (Khan et al. 2014a). Increased mineral excretion from the body and decreases in the blood is a major consequence of heat stress, leading to deficiencies of these components in blood and tissues (Sahin et al. 2009). Heat stress may also increase the mortality rate which leads to economic losses (Khan et al. 2011). The reduced feed consumption and the increased excretion of minerals have adverse effects on poultry performance, health, and antioxidant status (Khan et al. 2012; Chand et al. 2016). Moreover, in response to chronic heat stress, an increase in intracellular reactive oxygen species (ROS) was produced and this resulted in lipid hyperoxidation (Zuo et al. 2017). Many attempts have been tried to minimize the effect of heat stress by changing the environment and diets of laying hens. Cool poultry houses are usually expensive, so methods are focused mainly on nutritional modifications. It has been reported that some minerals such as Cr (Moeini et al. 2011; Ghazi et al. 2012) and Mg (Sahin et al. 2005a; Yang et al. 2012) can be supplemented to reduce the negative effects of heat stress.

Magnesium is one of the most abundant cations in living cells, is involved as a cofactor or an activator of many critical enzymes for the reactions involving ATP that energize all major metabolic pathways (Morii 2007). It is well known that Mg2+ deficiency contributes to the generation of ROS and related oxidative damage which can be reversed by Mg2+ supplementation in a series of human diseases. However, the molecular mechanisms underlying this observation are not well known (Chakrabarti et al. 2002). Liu et al. (2007) postulated that Mg may influence lipid peroxidation in the muscles of broiler chickens partly through affecting reactive oxygen species production. Accordingly, Mg deficiency induces an increase in hydrogen peroxide production and decreases catalase activity in chick embryo hepatocytes in vitro, indicating a link between magnesium deficiency and the oxidative damage in broilers (Yang et al. 2006). It has been showed that Mg supplementation may alleviate the detrimental consequences of stress in animals (Sahin et al. 2005a; Yang et al. 2012), so in stress conditions the requirement may be increased.

Trivalent chromium is associated with the metabolism of carbohydrates, lipids, and proteins in animals termed as ‘glucose tolerance factor’ since Cr regulates the metabolic action of insulin (Khan et al. 2014b). Cr improves insulin effectiveness by enhancing its binding to receptors and thus increasing the sensitivity of the target cell (Chen et al. 2012). Because insulin influences lipid peroxidation (Cole et al. 2008), Cr is considered to function as an antioxidant. Cr is also shown to be essential for the activating certain enzymes and stabilizing proteins (Hayirli 2005). The requirement for Cr may be increased mainly due to reduced feed intake (FI) and increased Cr excretion during heat stress (Sahin et al. 2005b; Sahin et al. 2010). Moreover, recent studies have obtained equivocal results in improved performance (Eseceli et al. 2010; Habibian et al. 2013) and serum insulin and glucose concentrations
(Habibian et al. 2013) when birds fed supplemental dietary chromium under either thermoneutral or heat stress conditions. In addition, organic Cr has been found to have more beneficial effects in heat-stress birds as compared to inorganic forms due to its increased absorption and bioavailability (Moeini et al. 2011). Benefits of dietary supplemental Cr in laying hens reared under cold or heat stress have been reported (Sahin et al. 2002a, 2002b).

The current study was conducted to evaluate the individual effects and probable synergistic interaction between the diet supplementation by Mg and Cr 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. A total of 324 Lohmann LSL Lite laying hens (30 wk age, with mean body weight of 1410 ± 140 g) were randomly allocated to 54 cages with six birds each. Birds in each six replicate cages were randomly allotted to one of the nine dietary treatments. 16 h of light/8 h dark was provided per day. The birds were reared under normal temperature (18 ± 2°C) for 11 weeks; then the temperature was increased gradually up to 31°C for 5 weeks. During the heat stress, 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%. Feed was offered on the basis of catalog (110 g/hen/day), and water (18 ± 4°C) was supplied ad libitum. A corn-soybean meal basal diet (metabolizable energy, ME = 2720 kcal/kg and crude protein, CP = 16.82 g/100 g diet) as formulated to contain adequate levels of all nutrients as recommended by the Lohmann LSL-Lite catalog (2011), except for Mg and Cr, in which their analysed value of the basal diet was 25.6 mg kg⁻¹ and 5.43 μg kg⁻¹respectively. The ingredients and composition of the basal diet are shown in Table 1. The birds were fed either a basal diet supplemented with 0, 300 and 600 mg of Mg kg⁻¹ of diet or 0, 400 and 800 μg of Cr kg⁻¹ of diet. Mg oxide (MgO) was used as the Mg source and Cr methionine, (CrMet) as Cr source. Small amounts of the basal diet were first mixed with the calculated amounts of Mg and Cr as a small batch and then with a larger amount of the basal diet until the total amount of the experimental diets were homogeneously mixed and each diet was randomly allocated to hens in every six replicate cages.

Productive performance and egg quality

The productive performance of the laying hens including EP, FI, and egg weight (EW) was recorded daily, from each cage, and feed conversion ratio (FCR) (g feed: g egg) and egg mass were calculated. To measure egg quality characteristics including yolk colour, shell thickness, shell weight, egg index, yolk index, yolk weight, albumen weight and Haugh unit, random samples of 36 eggs from each treatment, Prior to the heat stress (week 41) and during the heat stress (week 46), were collected. Eggs were broken and yolk was separated from albumen, then yolk weight and albumen weight was measured. Length and width of egg were individually recorded by using a compass. Egg index, then, was calculated by (width/length) × 100. Haugh unit was calculated using the Haugh unit formula (Eisen et al. 1962). The unit based on the height of albumen was determined by a micrometer and EW. Shell thickness was a mean value of measurement of three locations on the egg (air cell, equator, and sharp end) by using a dial and pipe gage. Yolk colour was measured using the Roche fan scale. Specific gravity of eggs was determined by using saline flotation method (Hempe et al. 1998). NaCl salt solutions were made in incremental concentrations of 0.005 in the range of 0.1065–0.1120 g/L. Yolk height (H) was measured by a tripod micrometer (Mitutoyo, 0.01 mm, Japan) and yolk diameter (D) by a compass (Swordfish, 0.02 mm, China), then the yolk index was calculated with the following formula [YI = (H/D) × 100] (Wells 1968).

Blood biochemical parameters

Week 41 of age (prior to the heat stress) and 46 (during the heat stress), blood samples (3.0 ml) were collected from the bronchial wing vein of six randomly selected 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 (1008 g) for 10 min, and the sera and plasma was frozen at −20°C until the analysis. Serum samples were thawed at room temperature and were analysed for glucose, total protein, triglyceride, cholesterol, albumin, uric acid (Pars Azmunkits, Tehran, Iran) and insulin (Immulate 2000, L2kin6, Dpc, Los Angeles, CA), besides, the enzyme activity of plasma glutathione peroxidase (GSH-Px) was measured.
Statistical analysis

The data were subjected to ANOVA in a completely randomized design with a 3x3 factorial arrangement of treatments using GLM procedure of SAS (SAS Institute 2003). All statements of significance are based on a probability of <0.05. The mean values were compared by Duncan’s multiple-range test (Duncan 1955). The following model was considered for analysis: Yijk = μ + (Mi) + (Cj) + (MCij) + (eijk); where Yijk is the measured characteristic, μ is the overall mean, (Mi) is the main effect of Mg, (Cj) is the main effect of Cr, (MCij) is the interaction between Mg and Cr, and (eijk) is the residual error. The effects of the main factors were not considered, whenever the interaction was significant.

Results

Performance production

The effects of dietary supplemental Mg and Cr on the performance of laying hens are shown in Tables 2 and 3. FI and EP were not affected by dietary supplemental Mg and Cr (P > 0.05). Improved FCR was seen in the birds fed the diet of 300 or 600 mg/kg supplemental Mg under normal temperature (30–41 wk), heat stress condition (41–46 wk) and overall period of the experiment (P < 0.05). A significant effect of dietary supplemental Cr on EW was detected during 30–41 wk, so that the birds fed 800 μg/kg Cr had the highest EW compared to those fed the basal diet (P < 0.05). Increased egg mass was seen in hens fed diet of 300 or 600 mg/kg supplemental Mg during normal temperature (30–41 wk), heat-stressed condition (41–46 wk) and overall period of the experiment (P < 0.05).

Blood parameters

The effects of dietary supplemental Mg and Cr on the blood biochemical parameters of hens are presented in Tables 7–9. Dietary supplemental Cr and Mg had no significant effects on the serum concentrations of total protein and the activity of GSH-Px (P > 0.05). Significant interactions between dietary supplemental Cr and Mg on serum concentration of insulin and albumin was detected (P < 0.05); laying hens fed the diet of the highest levels of Mg and Cr (the combined form) showed the lower serum level of insulin (P < 0.05). Serum concentration of albumin was significantly higher in the hens fed the diet supplemented by the combined form of Mg and Cr compared with the basal diet (P < 0.05). The main effects of dietary treatments were not discussed whenever the interaction between experimental factors (Mg and Cr) was significant. Under the heat stress conditions

Table 2. Effects of dietary supplementation by Mg (mg/kg) and/or Cr (μg/kg) on feed intake (g/hen/day), egg production (%) and Feed conversion ratio (g egg/g feed) of laying hens reared under thermo-neutral (30–41 weeks of age) and heat stress conditions (41–46 weeks of age).

| Heat stress Treatments | Feed intake (g/hen/day) | Egg production (%) | Feed conversion ratio (g egg/g feed) |
|------------------------|-------------------------|--------------------|----------------------------------|
|                        | Weeks 30–41              | Weeks 41–46        | Weeks 30–46                      |
|                        | Before heat stress       | During heat stress | Total period                     |
|                        |                         |                    |                                 |
|                        |                        |                    |                                 |
| Mg (mg/kg)             |                         |                    |                                 |
| 0                      | 109.84                  | 109.24             | 109.54                           |
| 300                    | 109.74                  | 108.94             | 109.34                           |
| 600                    | 109.65                  | 109.24             | 109.44                           |
| Cr(μg/kg)              |                         |                    |                                 |
| 0                      | 109.73                  | 109.00             | 109.37                           |
| 400                    | 109.76                  | 109.05             | 109.41                           |
| 800                    | 109.73                  | 109.37             | 109.55                           |
| SEM                    | 0.033                   | 0.076              | 0.041                            |
| CV                     | 0.24                    | 0.55               | 0.30                             |
| Mg × Cr                | 0.015                   | 0.245              | 0.218                            |
| Cr                     | 0.924                   | 0.153              | 0.223                            |
| Mg × Cr                | 0.681                   | 0.977              | 0.846                            |
| SEM                    | 0.033                   | 0.076              | 0.041                            |
| CV                     | 0.24                    | 0.55               | 0.30                             |
| P value                |                         |                    |                                 |

Means ±SD within columns with different lower case letters differ significantly (P < 0.05).

SEM standard error of the mean for main effects.

Egg quality

The effects of dietary supplemental Mg and Cr on the egg quality traits during thermo-neutral and heat stress condition are shown in Tables 4–6. No significant effect of dietary treatments was detected on Haugh unit, specific gravity, shell weight, yolk weight and yolk colour. Significant interactions between dietary supplemental Cr and Mg on yolk index and albumen weight in the first sampling (before heat stress), and egg index in the first and the second sampling (before and after heat stress) were detected (P < 0.05). The highest albumen weight was seen in the hens fed diet included 600 mg/kg supplemental Mg and 800 μg/kg Cr; however, the differences with the basal diet were not significant. The birds fed basal diet showed significantly higher egg index compared with hens fed the diet supplemented by Cr and Mg, as the single or combined forms (P < 0.05). Increased shell thickness was seen in hens fed the diet of 600 mg/kg supplemental Mg during heat stress condition (41–46 wk).
### Table 3. Effects of diet supplementation by Mg (mg/kg) and/or Cr (μg/kg) on egg weight (g) and egg mass (g/hen/day) of laying hens reared under thermo-neutral (30–41 weeks of age) and heat stress conditions (41–46 weeks of age).

| Heat stress Treatments | Age of sampling | Egg weight (g) | Egg mass (g/hen/day) |
|------------------------|----------------|---------------|---------------------|
|                        | 30–41          | 41–46         | 30–41               | 41–46               |
| Mg (mg/kg)             | Before heat    | During heat   | Total               | Before heat         | During heat         | Total               |
| 0                      | 59.28          | 58.83         | 59.14               | 56.67               | 54.46               | 55.82               |
| 300                    | 59.06          | 58.27         | 58.86               | 57.89               | 55.83               | 57.23               |
| 600                    | 59.66          | 58.88         | 59.42               | 58.05               | 56.01               | 57.36               |
| Cr (μg/kg)             | Before heat    | During heat   | Total               | Before heat         | During heat         | Total               |
| 0                      | 58.84          | 58.35         | 58.69               | 56.99               | 55.16               | 56.30               |
| 400                    | 59.39          | 58.57         | 59.19               | 57.72               | 54.42               | 56.88               |
| 800                    | 59.79          | 59.07         | 59.56               | 57.89               | 55.72               | 57.22               |
| Mg × Cr                | 0.013          | 0.159         | 0.140               | 0.155               | 0.243               | 0.172               |

Means (±SD) within columns with different lower case letters differ significantly (P < 0.05).

### Table 4. Effects of diet supplementation by Mg (mg/kg) and/or Cr (μg/kg) on Haugh unit, shell weight (g) and shell thickness (×10⁻³mm) of laying hens reared under thermo-neutral (30–41 weeks of age) and heat stress conditions (41–46 weeks of age).

| Heat stress Treatments | Age of sampling | Haugh unit | Shell weight (g) | Shell thickness (×10⁻³mm) |
|------------------------|----------------|-----------|-----------------|---------------------------|
|                        | 30–41          | Week 41   | Week 46         | Week 41                   | Week 46                   |
| Mg (mg/kg)             | Before heat    | During heat | Total          | Before heat         | During heat         | Total          |
| 0                      | 71.95          | 68.52     | 5.94            | 5.56                  | 0.37                 | 0.34             |
| 300                    | 72.18          | 69.35     | 5.94            | 5.59                  | 0.37                 | 0.35             |
| 600                    | 72.67          | 69.79     | 5.93            | 5.72                  | 0.37                 | 0.35             |
| Cr (μg/kg)             | Before heat    | During heat | Total          | Before heat         | During heat         | Total          |
| 0                      | 71.09          | 69.20     | 5.89            | 5.63                  | 0.37                 | 0.35             |
| 400                    | 72.50          | 68.64     | 5.91            | 5.62                  | 0.37                 | 0.35             |
| 800                    | 73.20          | 69.82     | 6.01            | 5.61                  | 0.37                 | 0.35             |
| Mg × Cr                | 0.037          | 0.038     | 0.024           | 0.034                 | 0.0014              | 0.0011           |

Means (±SD) within columns with different lower case letters differ significantly (P < 0.05).

### Table 5. Effects of diet supplementation by Mg (mg/kg) and/or Cr (μg/kg) on Egg gravity, yolk colour and yolk weight (g) of laying hens reared under thermo-neutral (Weeks 41 of age) and heat stress conditions (Weeks 46 of age).

| Heat stress Treatments | Age of sampling | Egg gravity | Yolk colour | Yolk weight (g) |
|------------------------|----------------|-------------|-------------|----------------|
|                        | Week 41        | Week 46     | Week 41     | Week 46        |
| Mg (mg/kg)             | Before heat    | During heat | Before heat | During heat    |
| 0                      | 74.21          | 72.74       | 40.97       | 37.69          |
| 300                    | 74.49          | 74.17       | 40.65       | 38.20          |
| 600                    | 73.98          | 74.44       | 40.74       | 38.12          |
| Cr (μg/kg)             | Before heat    | During heat | Before heat | During heat    |
| 0                      | 74.33          | 74.18       | 41.20       | 38.34          |
| 400                    | 74.22          | 73.86       | 40.47       | 38.21          |
| 800                    | 74.12          | 73.31       | 40.69       | 37.46          |
| Mg × Cr                | 0.113          | 0.176       | 0.182       | 0.158          |

Means (±SD) within columns with different lower case letters differ significantly (P < 0.05).

### Discussion

**Performance**

In the present study, improved FCR and egg mass was seen in the birds given either 300 or 600 mg/kg Mg as a single dietary supplement during thermoneutral condition as well as

### Table 6. Effects of diet supplementation by Mg (mg/kg) and/or Cr (μg/kg) on egg index (%), yolk index (%) and albumen weight (g) of laying hens reared under thermoneutral (Weeks 41 of age) and heat stress conditions (Weeks 46 of age).

| Heat stress Treatments | Age of sampling | Egg index (%) | Yolk index (%) | Albumen weight (g) |
|------------------------|----------------|--------------|---------------|-------------------|
|                        | Week 41        | Week 46      | Week 41       | Week 46           |
| Mg (mg/kg)             | Before heat    | During heat  | Before heat   | During heat       |
| 0                      | 74.21          | 72.74       | 40.97        | 37.69             |
| 300                    | 74.49          | 74.17       | 40.65        | 38.20             |
| 600                    | 73.98          | 74.44       | 40.74        | 38.12             |
| Cr (μg/kg)             | Before heat    | During heat  | Before heat   | During heat       |
| 0                      | 74.33          | 74.18       | 41.20        | 38.34             |
| 400                    | 74.22          | 73.86       | 40.47        | 38.21             |
| 800                    | 74.12          | 73.31       | 40.69        | 37.46             |
| Mg × Cr                | 0.113          | 0.176       | 0.182        | 0.158             |

Means (±SD) within columns with different lower case letters differ significantly (P < 0.05).

SEM standard error of the mean for main effects.
heat stress condition. Results from this study also indicated that the supplementation of Mg and Cr had no effect on FI and EP of laying hens, although increased EW was seen in the birds given 800 μg/kg Cr during thermoneutral condition. Similar to results of the present study, Atteh and Leeson (1983) found improved FCR in broiler chicks by increasing water Mg concentration (0, 25, 50, 75, or 100 ppm Mg). In addition, Sahin et al. (2005a) reported an increased FI, BW, and improvements in feed efficiency in heat-stressed quails fed either 1 or 2 g of Mg propionate or MgO. Kim et al. (2013) reported no effect on FI and EP of laying hens fed diets contained 150 mg/kg Cr from Cr yeast. In contrast to our results, Sahin et al. (2002b) reported an improved body mass, FI, EP, EW and FCR in the heat-stressed laying Japanese quails fed the Cr-supplemented diet (200, 400, 800, or 1200 μg/kg of diet as Cr picolinate) compared with birds fed the control diet. In additions, Sahin et al. (2010) reported that Cr picolinate (400 and 800 μg kg⁻¹) improved performance again in Japanese quails exposed to high ambient temperatures (34°C). The reason for the discrepancy between the results of various investigations can be partly due to several variables such as Cr status of the birds, birds’ age, Cr bioavailability of dietary ingredients, bioavailability of supplemental Cr, degree of stress, supplemented level, dietary composition, and duration of usage of supplemental Cr that may influence the efficacy of dietary supplemental Mg and/or Cr on EP, egg mass, FCR and BW in the laying hens reared under heat stress conditions.

| Table 7. Effects of diet supplementation by Mg (mg/kg) and/or Cr (μg/kg) on glucose (mg/dL), triglycerides (mg/dL) and cholesterol (mg/dL) of laying hens reared under thermoneutral (Weeks 41 of age) and heat stress conditions (Weeks 46 of age). |
|---|---|---|---|---|---|---|
| Age of sampling Heat stress Treatments | Glucose (mg/dL) | Triglycerides (mg/dL) | Cholesterol (mg/dL) |
| | Week 41 | Week 46 | Week 41 | Week 46 | Week 41 | Week 46 |
| Mg (mg/kg) | | | | | | |
| Before heat stress | During heat stress | Before heat stress | During heat stress | Before heat stress | During heat stress |
| 0 | 225.11 | 245.44 | 1255.32 | 1356.00 | 264.16 | 322.88 |
| 300 | 230.35 | 241.94 | 1204.62 | 1262.89 | 230.41 | 300.35 |
| 600 | 222.66 | 230.77 | 1158.96 | 1277.23 | 234.29 | 284.53 |
| Cr (μg/kg) | | | | | | |
| 0 | 234.94 | 248.33 | 1264.28 | 1398.25 | 289.20 | 336.01 |
| 400 | 227.44 | 240.05 | 1188.33 | 1292.48 | 247.72 | 311.68 |
| 800 | 216.00 | 229.77 | 1143.86 | 1218.75 | 259.92 | 315.92 |
| SEM | 1.71 | 2.54 | 24.65 | 17.88 | 8.30 | 8.30 |
| CV | 6.00 | 8.48 | 14.38 | 9.78 | 24.58 | 21.26 |
| Mg × Cr | 0.996 | 0.938 | 0.951 | 0.817 | 0.722 | 0.774 |
| Mg | 0.199 | 0.088 | 0.420 | 0.203 | 0.399 | 0.236 |
| Cr | 0.0006 | 0.030 | 0.221 | 0.004 | 0.0004 | 0.006 |
| Mg × Cr | 0.96 | 0.938 | 0.951 | 0.817 | 0.722 | 0.774 |

Means (±SD) within columns with different lower case letters differ significantly (P < 0.05).

Consistent with our findings, Torki et al. (2013) found no significant effect of dietary supplemental organic Cr on EP, egg mass, FCR and BW in the laying hens reared under heat stress conditions. In addition, Eseseli et al. (2010) reported no difference in EP, egg mass and FCR in laying hens fed diets contained 150 mg/kg Cr from Cr yeast. In contrast to our results, Sahin et al. (2002b) reported an increased body mass, FI, EP, EW and FCR in the heat-stressed laying Japanese quails fed the Cr-supplemented diet (200, 400, 800, or 1200 μg/kg of diet as Cr picolinate) compared with birds fed the control diet. In additions, Sahin et al. (2010) reported that Cr picolinate (400 and 800 μg kg⁻¹) improved performance again in Japanese quails exposed to high ambient temperatures (34°C). The reason for the discrepancy between the results of various investigations can be partly due to several variables such as Cr status of the birds, birds’ age, Cr bioavailability of dietary ingredients, bioavailability of supplemental Cr, degree of stress, supplemented level, dietary composition, and duration of usage of supplemental Cr that may influence the efficacy

| Table 8. Effects of diet supplementation by Mg (mg/kg) and/or Cr (μg/kg) on total protein (g/dL), uric acid (mg/dL), GSH-Px enzyme activity (μ/lit) and serum magnesium and chromium of laying hens reared under thermo-neutral (Weeks 41 of age) and heat stress conditions (Weeks 46 of age). |
|---|---|---|---|---|---|---|
| Age of sampling Heat stress Treatments | Total protein (g/dL) | Uric acid (mg/dL) | GSH-Px enzyme activity (μ/lit) | Mg(mg/dL) | Cr(μg/dL) |
| | Week 41 | Week 46 | Before heat stress | During heat stress | Week 41 | Week 46 | Before heat stress | During heat stress |
| Mg (mg/kg) | | | | | | | | |
| Before heat stress | During heat stress | Before heat stress | During heat stress | Before heat stress | During heat stress |
| 0 | 6.44 | 6.28 | 7.76 | 9.34 | 721.79 | 4.20 | 0.52 |
| 300 | 6.33 | 6.93 | 6.95 | 6.54 | 811.05 | 4.87 | 0.73 |
| 600 | 5.68 | 6.29 | 8.17 | 6.99 | 832.78 | 5.32 | 0.61 |
| Cr (μg/kg) | | | | | | | | |
| 0 | 6.26 | 7.16 | 8.26 | 10.00 | 802.96 | 4.66 | 0.30 |
| 400 | 5.72 | 6.02 | 7.15 | 6.54 | 782.31 | 4.94 | 0.58 |
| 800 | 6.47 | 6.31 | 7.36 | 6.52 | 777.87 | 4.79 | 0.97 |
| SEM | 0.223 | 0.207 | 0.387 | 0.264 | 0.49 | 0.047 | 0.043 |
| CV | 28.98 | 25.46 | 37.52 | 37.72 | 42.37 | 6.68 | 47.25 |
| P value | | | | | | | | |
| Mg | 0.393 | 0.409 | 0.436 | 0.014 | 0.725 | 0.0001 | 0.232 |
| Cr | 0.435 | 0.109 | 0.398 | 0.001 | 0.990 | 0.111 | 0.0001 |
| Mg × Cr | 0.316 | 0.386 | 0.239 | 0.069 | 0.233 | 0.055 | 0.644 |

Means (±SD) within columns with different lower case letters differ significantly (P < 0.05).

SEM standard error of the mean for main effects.
of Cr application, and thus, it was difficult to directly assess different studies using Cr.

**Egg quality**

During the heat stress, the higher shell thickness was detected in the hens fed diets supplemented with Mg compared with the control birds. Consistent with the present findings, Kim et al. (2013) reported that feeding 46-week-old laying hens with diets containing more than 4.2 g/kg Mg increased eggshell strength and shell thickness. Similar improvement in eggshell quality was also observed by Seo et al. (2010) who fed 26-week-old laying hens with diets containing 4.7 g/kg Mg. The reason for this improvement has been associated with increased concentration of Mg in egg shells, because Mg is the mineral with the second highest concentration in eggshells (Kim et al. 2013).

There were no significant effects of dietary treatments of Haugh unit, specific gravity, shell weight, yolk weight and yolk colour. These results are in accordance with those of Torki et al. (2013) who reported that adding organic Cr to the diet of laying hens reared under heat stress conditions had no significant effect on Haugh unit, egg index, shell weight and yolk colour. In addition, Kim et al. (2013) found no significant effect of increasing dietary Mg from 1.6 to 3.0 g/kg on yolk colour and Haugh unit in laying hens.

Diet supplementation in the combined form of Cr and Mg caused increased albumen weight with the highest albumen weight in the hens fed with 600 mg/kg and 800 µg/kg supplemental Mg and Cr, respectively. Uyanik et al. (2002b) indicated that dietary supplementation with 20 ppm Cr from Cr chloride increased albumen weight in laying hens. In addition, Yildiz et al. (2004) reported linearly increased yolk weight and albumen weight as dietary Cr level increased. The mechanism by which Cr and Mg exerts its effect on albumen weight is not known with certainty. However, according to Hossain et al. (1998), Cr augments egg quality by acting as a structural component of egg albumin or in cross-linking of protein, necessary for the synthesis of ovomucin and facilitates the transfer of cation into the albumin of egg during the plumping process in the uterus. In addition, the effect of Mg on albumen weight found in the present study might be partly due to the Mg function of energy metabolism and protein synthesis (Morii 2007).

**Blood parameters**

Lower serum concentrations of glucose, total cholesterol and triglycerides were seen in the birds fed the Cr-supplemented diet compared with basal diet, but dietary Mg supplementation did not have significant effect on the blood biochemical. Cr has been well demonstrated as the active component in GTF, which promotes the sensitivity of tissue receptors to insulin, resulting in increased glucose uptake by cells. In addition, insulin regulates carbohydrate and fat metabolism, protein synthesis and glucose utilization in tissues (Chen et al. 2012; Khan et al. 2014a). Thus the decreased serum concentrations of glucose may be a result of the enhanced sensitivity of tissue receptors to the insulin directed by Cr supplementation. Decrease in serum level of glucose following dietary supplementation with Cr picolinate has been also detected in broiler chickens, laying hens and Japanese quails (Sahin et al. 2001b; Uyanik et al. 2002a; Yildiz et al. 2004). The decreased serum concentration of triglycerides in the Cr-supplemented group could be explained by insulin action as anabolism stimulator and inhibitor in lipids catabolism, which in turn causes increase uptake of blood glucose by cells (Cupo and Donaldson 1987). On the other hand, increased glucose uptake is expected to increase glucose oxidation, which would otherwise be converted to fatty acids and stored as triglycerides in adipose tissues. Parallel to our results, Torki et al. (2013) found decreased serum concentration of glucose, triglycerides and cholesterol in laying hens fed diet supplemented with organic Cr during heat stress conditions. Similarly, Moeini et al. (2011) indicated lower serum concentration of triglycerides in broilers fed a diet supplemented with Cr methionine. In addition, Cupo and Donaldson (1987) detected an increased rate of glucose utilization following dietary Cr supplementation (20 mg/ kg of CrCl3.6H2O). Sahin et al. (2005b) showed the reducing effect of dietary supplemental Cr and biotin, alone or together, on serum levels of cholesterol and glucose in quails reared under thermo-neutral and heat stress conditions. The reduction of blood cholesterol may be due to an augmented insulin action that reduces lipolysis and increases the incorporation of fatty acids in the adipocytes (Vincent 2000; Khan et al. 2014a).

In this study no significant effect of dietary Mg was detected on glucose, total cholesterol and triglycerides. However, Sahin et al. (2005a) found that dietary Mg supplementation reduced serum triglyceride and cholesterol concentration in heat-

**Table 9.** Effects of diet supplementation by Mg (mg/kg) and/or Cr (µg/kg) on albumin (g/dL) and insulin (µlU/mL) concentration of laying hens reared under thermoneutral (Weeks 41 of age) and heat stress conditions (Weeks 46 of age).

| Age of sampling | Heat stress Treatments | Albumin (g/dL) | Insulin (µlU/mL) |
|-----------------|------------------------|----------------|------------------|
|                 | Before heat stress     | Week 41        | During heat stress | Week 46 |
| Mg (mg/kg)      | 0                      | 30.18          | 28.58            | 30.66  |
|                 | 300                    | 29.70          | 33.62            | 13.52  |
|                 | 600                    | 28.92          | 33.76            | 19.07  |
| Cr (µg/kg)      | 0                      | 30.60          | 29.79            | 22.36  |
|                 | 400                    | 29.53          | 32.91            | 18.01  |
|                 | 800                    | 28.57          | 33.55            | 22.87  |
|                 | SEM                    | 0.656          | 0.588            | 0.974  |
|                 | CV                     | 16.73          | 14.52            | 31.58  |

P value

| Mg | 0.724 | 0.003 | 0.0001 |
| Cr | 0.503 | 0.053 | 0.164  |
| Mg × Cr | 0.374 | 0.007 | 0.0001 |

| Mg × Cr | 33.05 | 24.35 | 9.26  |
| 0 × 400 | 30.92 | 30.95 | 40.20 |
| 0 × 800 | 26.95 | 30.83 | 42.51 |
| 3000 | 30.30 | 33.89 | 24.82 |
| 3000 × 400 | 30.28 | 29.87 | 3.98  |
| 3000 × 800 | 28.52 | 37.11 | 11.75 |
| 6000 | 28.40 | 31.14 | 33.01 |
| 6000 × 400 | 27.95 | 37.90 | 9.84  |
| 6000 × 800 | 30.25 | 32.25 | 14.35 |

Means (±SD) within columns with different lower case letters differ significantly (P < 0.05).

SEM standard error of the mean for main effects.
stressed Japanese quail. According to report by Kucuk (2008) in quails reared under heat stress condition (35°C), diet supplementation by both Zn and Mg (in a combined form) decreased the serum content of glucose compared with the birds fed the diets added with Zn or Mg separately. Moreover, dietary supplemental Mg and Zn (separately) increased the serum content of triglycerides. The lack of concurrence among these studies may be partially explained by variables such as birds’ age, bioavailability of supplemental Mg, stress condition, degree of stress, supplemented level, dietary composition, and duration of usage of supplemental Mg.

In our study, there was no significant effect of diet supplementation by Cr and Mg on the serum content of total protein. However, increased serum content of albumin was seen in hens fed the diet supplemented by Cr and Mg (in the combined form) compared to the birds fed the basal diets during heat stress condition. These results revealed additive effects of Mg and Cr, indicating that Mg and Cr work together or act synergistically. Chen et al. (2001) reported no significant influence of dietary supplemental Cr (1–3 mg/kg) on serum total proteins in male turkey. In addition, Kucuk (2008) reported that dietary supplemental Mg had no effect on protein concentration on heat-stressed Japanese quail. Sahin et al. (2003) found the increased serum levels of protein in broiler chickens fed Cr supplemented diet in a high ambient temperature. In our study, the effect of the combined form of Mg and Cr on increasing the serum content albumin may be partly due to a synergistic interaction. The increase in serum albumin concentrations by supplemental Cr may be due to an increased synthesis of amino acids in the liver via insulin which enhances the incorporation of several amino acids into protein (Moeini et al. 2011). In addition, the effect of Mg on serum albumin concentration found in the present study might be due to the Mg function of energy metabolism and protein synthesis (Mori 2007).

Lower serum concentrations of uric acid in laying hens fed the diets supplemented by Cr or Mg was observed during heat stress. Increased circulating corticoids occurring under stress conditions has suppressed effect on synthesis of body proteins and enhanced proteolysis, because these hormones exert mainly catabolic effects. Degradation of proteins is supposed to produce uric acid. Arad et al. (2006) observed a significant increase in the level of uric acid in chickens exposed to heat stress. In contrast, Akbari and Torki (2013) reported no significant effect of dietary supplemental Cr on serum concentration of uric acid in heat-stressed broiler chickens. The exact mechanism for this action is not clear, but chromium is thought to be essential for activating certain enzymes and for stabilizing proteins and nucleic acids (Khan et al. 2014a). We have found no study showing the action mechanism of Mg on serum uric acid in animals in the literature to compare our result. But the reduced blood level of uric acid in laying hens fed the diet supplemented with Mg might be partly due to the role of Mg in protein metabolism (Mori 2007). Moreover, there was a study showing an inverse relationship between Mg deficiency and the increased serum uric acid level among 94 diabetic retinopathy patients, supporting a potential role of Mg in the prevention of Hyperuricemia and reducing blood uric acid (Navin et al. 2013).

In the present study, the serum concentration of Cr and Mg increased due to the increasing levels of dietary Cr and Mg respectively. Similarly, increased serum concentrations of Cr were detected in laying hens fed diets supplemented by both organic and inorganic Cr (Ghazi et al. 2012). Uyanik et al. (2002a) stated that Cr supplementation increased serum concentrations of Cr in chicks. In addition, Akbari and Torki (2013) reported that dietary supplemental Cr picolinate increased the plasma concentration of Cr. Based on the report on Ding and Shen (1992) in leghorn hens, the plasma Mg level was significantly increased from 1.66 mg/dL to 4.03 mg/dL following the increased dietary Mg content of 690–2380 mg/kg, suggesting that the plasma Mg concentration apparently influenced by the absorbed Mg in the intestine. Sahin et al. (2005a) suggested that dietary Mg supplementation increased serum concentration of Mg in heat-stressed Japanese quail. In contrast to our result, Kucuk (2008) reported that dietary Mg and Zn (separately) had no effect on serum concentration of Mg in heat-stressed Japanese quail.

The observations of the present study show that serum insulin content was gradually decreased when Mg and Cr were simultaneously supplemented, so the birds that fed on diet supplemented with the highest levels of the combined form of Mg and Cr, had serum insulin content similar to those of the basal diet, indicating the negative interactions between Mg and Cr on serum insulin. The interaction between Mg and Cr on serum insulin merits further research. The highest concentration of insulin was found the birds fed the diets supplemented with Cr or Mg alone. The metabolic relationship between blood levels of insulin and dietary Cr has been also demonstrated in other studies (Sahin et al. 2001b) indicating Cr’s physiologic role on insulin. Similar to the results of the present study, increased serum level of insulin in laying hens and Japanese quail after dietary supplementation with Cr has been also reported by Sahin et al. (2001a, 2004, 2005b). In addition, increased plasma concentration of insulin was detected in the heat-stressed broiler chickens fed the diet supplemented with Cr (Moeini et al. 2011). The important role of Mg in glucose homeostasis via influencing both insulin secretion and action has been also demonstrated (Mori 2007). It has been also reported that low intracellular Mg leads an impairment of insulin action and have a worsening effect on insulin resistance in hypertension and non-insulin-dependent diabetes mellitus (Mori 2007).

Dietary supplemental Cr and Mg had no significant effect on activity of plasma GSH-PX. Parallel to this result, Norouzi et al. (2013) reported that Mg does not influence plasma antioxidant status in broiler chickens reared under heat stress condition. There are evidences shows that Mg deficiency causes oxidative damage by decreasing enzymatic or nonenzymatic antioxidants (Yang et al. 2012). It has been thought that GSH-PX, among the antioxidant enzymes including superoxide dismutase, catalase and GSH-PX, is more effective in protecting cells, tissues and organs against oxidative damage by catalysing the reduction of H2O2 and preventing the formation of toxic radicals. Yang et al. (2012) suggested GSH-PX as the major responsible enzyme for the beneficial effect of Mg in revering heat stress-induced oxidative damage. Rama Rao et al. (2012) reported that GSH-PX activity elevated with increased dietary
Cr concentration in Broiler Chickens. In addition, Perai et al. (2014) detected the higher activity of GSH-Px in the transported broiler chickens fed diet supplemented with the vitamin C plus Cr compared with the control and the birds received supplemental Cr alone. The lack of concurrence among our study with these results may be partially explained by birds’ age, bioavailability of supplemental Mg and Cr, stress condition, degree of stress, supplemented level and dietary composition.

Conclusion

From the results of the present investigation, it can be concluded that dietary supplemental Mg has beneficial effects on FCR, egg mass and eggshell thickness of laying hens in normal as well as heat stress conditions. In addition, decreased serum concentrations of glucose, total cholesterol, and triglycerides were detected in the birds given dietary Cr supplement, whereas the significant interaction between dietary supplemental Mg and Cr on decreasing serum concentrations of uric acid was observed in the birds experienced heat stress condition. Moreover, increased serum concentration of albumen and decreased blood level of insulin were detected in the birds fed diet supplemented with both Mg and Cr. Overall, diet supplementation with Mg and Cr can be considered as a protective management practice in laying hens during heat stress condition.

Disclosure statement

The authors have no conflict of interest to declare.

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