Efficacy of dietary supplementation of nanoparticles-chromium, chromium-methionine and zinc-proteinate, on performance of Japanese quail under physiological stress

Arash Barzegar Yarmohammadi, Seyed Davood Sharifi and Abdollah Mohammadi-Sangcheshmeh
Department of Animal and Poultry Science, College of Aburaihan, University of Tehran, Tehran, Iran

ABSTRACT
The effects of dietary Nanoparticles-Chromium, Chromium methionine, and Zinc-proteinate on performance, blood characteristics and meat quality of physiologically stressed quails were studied. The stress was induced by adding dexamethasone (0.6 mg/kg BW) to diet when quails were between 17 and 22 days of age. Three hundred one-day chicks were randomly assigned to five groups comprising four replicates of 15 birds: negative control diet (no-stress, no-additive), positive control diet (stress, no-additive), and stress additive diets which included three diets containing 800 mg/kg of Nano-Cr, 800 mg/kg Cr-met, and 60 mg/kg Zn-pro. Performance and hematological parameters were affected by physiological stress ($p < .05$), but were not affected by dietary Nano-Cr, Cr-met or Zn-pro. During the stress period, feed consumption was improved by Zn-pro supplementation. By the end of the experiment, the ultimate pH value of the muscle in stressed birds, which were fed on the Cr-met diet, was higher than those treated with NC, but pH values were not affected by the treatments. Diets containing Nano-Cr and Zn-pro caused a reduction in malondialdehyde concentration in the thigh muscle. In summary, 800 mg/kg of Nano-Cr and 60 mg/kg of Zn-pro can reduce the negative effects of stress on meat quality.

HIGHLIGHTS
- Dietary dexamethasone induced physiological stress in quails.
- The supplementation of 60 mg/kg of Zinc proteinate had positive effect on feed intake of birds under physiological stress.
- Dietary Zinc proteinate and Nanoparticles-Chromium increased meat oxidative stability in birds which experienced physiological stress.

Introduction
Poultry farming is often confronted by various stressors such as vaccination, high stocking density, feed withdrawal, high and low ambient temperature, catching, crating, and transportation. Stressful conditions, whether acute or chronic, have various detrimental effects on the physiological features of birds, and such conditions can damage the production performance of poultry (Attia et al. 2016, 2018). Long-term exposure to chronic stress (CS) causes a loss in body weight (BW) and hampers weight gain (WG) (Wall and Cockrem 2010). It can reduce feed intake and feed efficiency (Gao et al. 2010), increase the catabolism of structural proteins (Puvadolpirod and Thaxton 2000b) and cause a decrease in liver weight and abdominal fat deposition (Virden and Kidd 2009).

Stress results in an atrophy of the lymphoid glands like thymus and bursa of fabricius in birds (Puvadolpirod and Thaxton 2000a; Sultana et al. 2020). Following lymphopenia and an increase in heterophil levels in the bloodstream, then, heterophil/lymphocyte ratio (H: L) usually increases in stressed birds (Aengwanich 2007). Physiological stress in farm animals causes several undesirable changes for meat quality. Some of these effects include cooking loss, the reduction of water-holding capacity (WHC), juiciness, high ultimate pH values (Ali et al. 2008). The available scientific literature suggests an increased urinary excretion of Zn (Sahin et al. 2006) and Cr (Ghazi et al. 2012) in stressed birds. Zinc is a necessary micronutrient element for animals because it is a cofactor of over 200 enzymes and has a key role in...
It is known that 25–50 mg Zn/kg of feed is normally required for the rearing of quails in standard circumstances (Korenkova et al. 2007). Also, Zn can participate in energy regulation, lipid metabolism, protein degradation and synthesis (Attia, Abd Al-Hamid, ElKomy, et al. 2013; Muralisankar et al. 2014). Japanese quails are relatively susceptible to Zn deficiency, compared to other poultry species, and it is known that 25–50 mg Zn/kg of feed is normally required for the rearing of quails in standard circumstances (Korenkova et al. 2007).

It is suggested that high ambient temperature can cause stress in quails which, in turn, reduce the retention of Cr in their bodies (Sahin et al. 2005). One of the main effects of Cr deficiency is the retardation in growth, as the activities of enzymes involved in protein synthesis become weak (Sahin et al. 2005). Therefore, it seems that beneficial effects of Cr are more noticeable under stressful conditions (Gultepe et al. 2018). Recently, the positive effects of mineral nanoparticles have been demonstrated, indicating that the Nano forms of minerals are more absorbable, bioavailable and effective (Ramiah et al. 2019). Nanoparticles can improve growth performance, meat quality, activities of the digestive system and metabolic or antioxidant enzymes, along with higher mineral contents in different tissues (Zha et al. 2009b; Muralisankar et al. 2014; Wang et al. 2014; Gangadoo et al. 2018).

In previous experiments, which aimed at inducing physiological stress, some stress hormones such as corticosterone dissolved in drinking water (Wall and Cockrem 2010; Nazar et al. 2012) and diet (Virden et al. 2009, 2007) or were used in injections of corticosterone or Adrenocorticotropic hormone (ACTH) (Virden et al. 2007; Luo et al. 2013). In addition to these hormones, corticosteroid analogs such as dexamethasone (DEX, a synthetic glucocorticoid exhibiting high affinity for glucocorticoid receptors) have been used in many studies to simulate the effects of glucocorticoid and stress induction (Adedapo et al. 2004; Li et al. 2009; de Lv et al. 2018).

Here, we hypothesised that the provision of dietary supplementation with Zn or Cr may reduce the negative effects of stressors in the poultry industry. We were also interested in evaluating the effects of Cr in its different physical forms, nanoparticles (Nano-Cr) or Cr-methionine (Cr-met) when incorporated into poultry diets under stress conditions. The dietary intake of DEX was used in this study to induce physiological stress in Japanese quail. Previous studies have confirmed that supplemental different trace minerals such as Zn and Cr can alleviate negative effects on growth development, hematological parameters, and immunological traits caused by heat stress in poultry species (Sahin et al. 2006; Sahin et al. 2010; Attia, Abd Al-Hamid, ElKomy, et al. 2013). However, under the condition of physiological stress, available information about comparing between various sources of Cr and Zn on meat quality, live performance, hematological parameters, and weight of internal organs of quails are lacking. Therefore, this study focussed on the effects of two different sources of Cr (i.e. Cr-nanoparticles and Cr-methionine) and Zinc-proteinate as supplements in the diet, the effects of mentioned minerals were examined on growth performance, carcass composition, immune response and meat quality of the Japanese quails which experienced physiological stress induced by the dietary intake of DEX.

**Materials and methods**

All experimental procedures on the birds in the present study were conducted in accordance with guidelines of the Animal Care Committee of Animal Science Research, University of Tehran, Iran.

**Birds, management and treatments**

On the day of their hatching, a total number of 300 unsexed Japanese quail chicks (Coturnix japonica) were purchased from a local hatchery and were reared in a sanitised room with a controllable environment. The chicks were fed on a basal diet based on corn and soybean meal for the first 16 days. The basal diet was formulated by using the NRC (1994). The diet contained 24% protein and 2850 kcal/kg ME. The ingredients and chemical analysis of the basal diets are shown in Table 1. The brooding temperature was maintained at 35 °C and humidity was at 55% for the first 3 days. Temperature and humidity were reduced gradually to 25 °C and 45% respectively until the chicks became 23 days old, and thereafter the temperature and humidity were stably maintained until the end of the experiment. All diets and fresh water were provided ad libitum. At 17 days of age, birds were randomly allocated into 20 cage pens (dimensions 70 × 30 × 50 cm) with the same mean value of BW (72.50 ± 3.43 g). They were randomly assigned to 1 of the 5 dietary treatments with 4 replicate cages, each containing 15 birds. Dexamethasone (0.6 mg/kg of BW) was administered in mash diet through time from the seventeenth to the twenty-second day in order to induce physiological stress over a period of six days. Each DEX pill contained 0.5 mg of dexamethasone.
Table 1. Ingredients and chemical analyses of the basal diet fed to quails under physiological stress.

| Item                          | Ingred. (% as fed) | Zinc of basal diet (mg/kg) | Chromium of basal diet (mg/kg) |
|-------------------------------|--------------------|---------------------------|-------------------------------|
| Maize                         | 48.64              |                           |                               |
| Soybean meal                  | 45.00              |                           |                               |
| Dicalcium phosphate           | 2.10               |                           |                               |
| Limestone                     | 1.90               |                           |                               |
| Soybean oil                   | 0.80               |                           |                               |
| Salt                          | 0.69               |                           |                               |
| Vitamin premixa                | 0.30               |                           |                               |
| Mineral premixb                | 0.30               |                           |                               |
| L-Lysine-HCL                   | 0.16               |                           |                               |
| DL-Methionine                  | 0.11               |                           |                               |
| Calculated composition        |                    |                           |                               |
| AMEn (% kcal/kg)              | 2850               |                           |                               |
| Crude protein (%)             | 24                 |                           |                               |
| Available phosphorus (%)      | 1.30               |                           |                               |
| Calcium (%)                   | 0.65               |                           |                               |
| Lysine (%)                    | 1.65               |                           |                               |
| Methionine (%)                | 0.52               |                           |                               |
| Methionine + Cystine (%)      | 0.91               |                           |                               |
| Determined composition        |                    |                           |                               |
| Chromium of basal diet (µg/kg)| 550.24             |                           |                               |
| Zinc of basal diet (mg/kg)    | 50.15              |                           |                               |

Vitamin premixa 0.30 Salt 0.69 Soybean oil 0.80 Limestone 1.90 Dicalcium phosphate 2.10

Ital J Anim Sci (2016) 15:1125


dietetic counts of white blood cells (WBCs). Leukocytes including granular (heterophils) and nongranular (lymphocytes and monocytes) were counted and the heterophil/lymphocyte ratio was calculated as a reliable stress index (Davis et al. 2008). The quantities and presence of monocytes, lymphocytes and heterophils were evaluated manually by using a Neubauer haemocytometer (Celeromics®, Spain). Moreover, the haemoglobin (Hb) concentration and haematocrit (Hct) values were measured by cyanmethemoglobin and by the microhematocrit method, respectively (Kececi et al. 1998).

**Blood and data collection**

Blood samples were collected in heparinised form or without anticoagulant tubes (Avapezeshk Company®, Tehran, Iran) and were then centrifuged at 1500 × g for 15 min at 4°C in order to perform diacritical counts of white blood cells (WBCs). Leukocytes including granular (heterophils) and nongranular (lymphocytes and monocytes) were counted and the heterophil/lymphocyte ratio was calculated as a reliable stress index (Davis et al. 2008). The quantities and presence of monocytes, lymphocytes and heterophils were evaluated manually by using a Neubauer haemocytometer (Celeromics®, Spain). Moreover, the haemoglobin (Hb) concentration and haematocrit (Hct) values were measured by cyanmethemoglobin and by the microhematocrit method, respectively (Kececi et al. 1998).

**Meat quality evaluation**

At the end of the stress and post-stress recovery periods, the entire right section of the pectoralis major (breast muscle) and the right Sartorius (thigh muscle) of quails were sampled in order to determine their pH values and WHC. The pH values of the breast and the
thigh muscle were determined by using an electronic portable pH metre (Metrohm® 827 pH lab, USA). The pH metre was used immediately after slaughter (pH0) and also on the sixth hour of the post-mortem era (ultimate pH or pHu). The pH metre was calibrated using standard buffer solutions with pH values of 4.0 and 7.0. The pH value was expressed as the average of the 3 measurements. Briefly, the procedure took 15 s as the electrode was inserted into the anterior portion of the muscle, and the penetration was approximately 2.5 cm deep.

Water-holding capacity of the breast and the thigh muscle of quails were estimated by centrifuging 1 g of the muscles placed on tissue paper inside a tube for 4 min at 1500 × g. The water remaining after centrifugation was quantified by drying the samples at 70 °C overnight. WHC was calculated by using the following formula (Castellini et al. 2002):

\[
\text{WHC} = \frac{(\text{weight after centrifugation–weight after drying}) \times 100}{\text{initial weight}}
\]

The assessment of lipid peroxidation as thiobarbituric acid reactive substances (TBARS) in muscles was performed by making slight modifications to the method reported by Botsoglou et al. (Botsoglou et al. 2002). Thigh meat samples were stored in a refrigerator at 4 °C for 5 days. Subsequently, they were centrifuged for 3 min at 3000 × g while being in mixture with aqueous trichloroacetic acid (4 ml) and butylated hydroxytoluene in hexane (2.5 ml). The top layer was discarded and a 2.5 ml aliquot from the bottom layer was mixed with 1.5 ml of aqueous thiobarbituric acid to be further incubated at 70 °C for 30 min. Following incubation, the mixture was cooled immediately with crushed ice and was then analysed by spectrophotometry (Perkin Elmer®, Lambda 25 UV, USA) at 521.5 nm. The values of TBARS materials were expressed in terms of malondialdehyde (MDA, mg/kg of meat).

Statistical analysis

Shapiro-Wilk tests were applied to assess all data for normality by the CAPABILITY procedure of SAS. If the confirmation indicated that this assumption could not be supported for an ANOVA, the data were transformed. Then, the percentage data were subjected to arcsine transformation in order to make them conform to data normality. All data were subjected to the one-way ANOVA procedures appropriate for a completely randomised design using the general linear model procedure of SAS (SAS Institute Inc., Cary, NC) according to the following model:

\[
Y_{ij} = \mu + T_j + e_{ij}
\]

Where \(Y_{ij}\) was the value of each observation, \(\mu\) was the overall mean, \(T_j\) was the effect of treatment, and \(e_{ij}\) is random errors. Differences among treatment means were determined using Tukey’s test and statistical differences declared at \(p < .05\).

Results

Growth performance

During the stress period, the quails that ingested DEX, Nano-Cr, and Cr-met had lower FI (\(p = .001\)) in comparison with those that ingested Zn-pro. Furthermore, all birds that received DEX had lower WG (\(p = .001\)) compared to the NC group over the stress period. During the stress period, supplementing the PC diet with Zn-pro increased the FI (\(p = .001\)), but had no significant effect on weight gain (or loss) and feed efficiency. Chromium supplementation, in its forms of Nano or chelated, had no effects on the parameters of live performance under the stress condition.

At the end of the stress period, the final body weight and weight gain of the stressed birds were significantly lower (\(p = .001\)) compared to the NC group. During the stress period, supplementing the PC diet with Zn-pro decreased parallel to feed efficiency, while the ratio of ME/WG and CP/WG increased (\(p < .001\)) in response to stress and dietary supplements during the entire experimental period (17–37 days) (Table 4). This
means that the stressed birds demanded more CP and ME in order to gain weight.

**Blood traits**

Hematological parameters showed differences among quails at 23 and 37 days of age (Tables 5 and 6). Generally, over the stress period the total count of White Blood Cells (WBC) on the twenty-third day was lower in stressed quails, and there was a significance difference \( (p = .021) \) between the unsupplemented PC group and the one supplemented with Cr-met. However, supplementing the PC with Zn-pro and Nano-Cr had no significant impact on the WBC during the stress period (Table 5). The concentrations of Hb and Hct were influenced by physiological stress \( (p = .041, p = .047) \) in the stressed group receiving the PC diet, but Hb and Hct values were not affected by experimental treatments (Table 5). The proportion of heterophils in the PC group was higher compared to that in the NC \( (p = .048) \). The lymphocyte percentage was significantly lower \( (p = .005) \) in the Cr-met group compared to the negative control. The ratio of heterophil to lymphocyte percentage in quails that received diets supplemented with Zn-pro and Nano-Cr was lower \( (p = .003) \) compared to those fed with PC. Monocytes percentage was not affected by physiological stress during the stress period.

At the end of the recovery period, birds of the PC group supplemented with Zn-pro showed a decrease in H: L ratio \( (p = .008) \). Hb and Hct values were not affected by experimental treatments (Table 6). The

---

**Table 2.** Effects of supplemental Nano-Cr, Cr-met and Zn-pro on performance of Japanese quail\(^1\) reared under physiological stress (stress period, 17–22 days).

| Treatment | NC | PC | Nano-Cr | Cr-met | Zn-pro | Pooled SEM | \( p \) Value |
|-----------|----|----|---------|--------|--------|------------|-------------|
| Initial weight (g) | 70.50 | 71.67 | 73.33 | 70.59 | 73.67 | 0.633 | .497 |
| Final weight (g) | 108.95\(^a\) | 63.13\(^b\) | 63.17\(^b\) | 64.01\(^b\) | 65.34\(^b\) | 0.570 | <.001 |
| Weight gain (g/day) | 6.42\(^b\) | –1.69\(^b\) | –1.64\(^b\) | –1.54\(^b\) | –1.66\(^b\) | 0.041 | <.001 |
| Feed Intake (g/day) | 15.51\(^b\) | 10.88\(^b\) | 11.41\(^b\) | 11.30\(^b\) | 12.24\(^b\) | 0.084 | <.001 |
| Feed efficiency\(^c\) | 0.414\(^a\) | –0.156\(^b\) | –0.143\(^b\) | –0.136\(^b\) | –0.135\(^b\) | 0.0044 | <.001 |

\(^a-b\)Mean values within a row with no common superscript differ significantly \( (p < .05) \).

NC: negative control (no-DEX, no-additive); PC: positive control (0.6 mg/kg BW DEX, no-additive), Nano-Cr (PC + 800 \( \mu \)g Nano-Cr/kg diet), Zn-pro (PC + 60 mg Zn-pro/kg diet), Cr-met (PC + 800 \( \mu \)g Cr-met/kg diet).

\(^1\)Data are means of four observations per diet.

\(^c\)Grams weight gained/g feed consumed.

---

**Table 3.** Effects of supplemental Nano-Cr, Cr-met and Zn-pro on performance of Japanese quail\(^1\) post-stress recovery period (23–37 days).

| Treatment | NC | PC | Nano-Cr | Cr-met | Zn-pro | Pooled SEM | \( p \) Value |
|-----------|----|----|---------|--------|--------|------------|-------------|
| Initial weight (g) | 70.50 | 71.67 | 73.33 | 70.59 | 73.67 | 0.633 | .497 |
| Final weight (g) | 108.95\(^a\) | 63.13\(^b\) | 63.17\(^b\) | 64.01\(^b\) | 65.34\(^b\) | 0.570 | <.001 |
| Weight gain (g/day) | 6.42\(^b\) | –1.69\(^b\) | –1.64\(^b\) | –1.54\(^b\) | –1.66\(^b\) | 0.041 | <.001 |
| Feed Intake (g/day) | 15.51\(^b\) | 10.88\(^b\) | 11.41\(^b\) | 11.30\(^b\) | 12.24\(^b\) | 0.084 | <.001 |
| Feed efficiency\(^c\) | 0.414\(^a\) | –0.156\(^b\) | –0.143\(^b\) | –0.136\(^b\) | –0.135\(^b\) | 0.0044 | <.001 |

\(^a-b\)Mean values within a row with no common superscript differ significantly \( (p < .05) \).

NC: negative control (no-DEX, no-additive); PC: positive control (0.6 mg/kg BW DEX, no-additive), Nano-Cr (PC + 800 \( \mu \)g Nano-Cr/kg diet), Zn-pro (PC + 60 mg Zn-pro/kg diet), Cr-met (PC + 800 \( \mu \)g Cr-met/kg diet).

\(^1\)Data are means of four observations per diet.

\(^c\)Grams weight gained/g feed consumed.

---

**Table 4.** Effects of supplemental Nano-Cr, Cr-met and Zn-pro on performance of Japanese quail\(^1\) in total experiment period (17–37 days).

| Treatment | NC | PC | Nano-Cr | Cr-met | Zn-pro | Pooled SEM | \( p \) Value |
|-----------|----|----|---------|--------|--------|------------|-------------|
| ME/WG (kCal/g) | 9.85\(^b\) | 17.00\(^a\) | 15.34\(^a\) | 15.25\(^a\) | 16.23\(^a\) | 0.522 | .012 |
| CP/WG (g/kg BW) | 0.90\(^b\) | 1.55\(^a\) | 1.40\(^a\) | 1.39\(^b\) | 1.48\(^b\) | 0.050 | <.001 |
| Weight gain (gr/day) | 5.57\(^b\) | 6.40\(^b\) | 6.55\(^a\) | 6.41\(^a\) | 6.58\(^a\) | 0.062 | .001 |
| Feed Intake (g/day) | 22.43\(^b\) | 19.84\(^b\) | 20.41\(^a\) | 20.93\(^b\) | 20.51\(^a\) | 0.201 | .021 |
| Feed efficiency\(^c\) | 0.25\(^b\) | 0.32\(^a\) | 0.32\(^a\) | 0.31\(^a\) | 0.32\(^a\) | 0.0041 | <.001 |

\(^a-b\)Mean values within a row with no common superscript differ significantly \( (p < .05) \).

NC: negative control (no-DEX, no-additive); PC: positive control (0.6 mg/kg BW DEX, no-additive), Nano-Cr (PC + 800 \( \mu \)g Nano-Cr/kg diet), Zn-pro (PC + 60 mg Zn-pro/kg diet), Cr-met (PC + 800 \( \mu \)g Cr-met/kg diet).

\(^1\)Data are means of four observations per diet.

\(^c\)Grams weight gained/g feed consumed.
percentage of monocytes was not affected by the physiological stress by the end of the recovery period.

**Meat quality**

The effects of dietary supplements on the qualitative parameters of the thigh muscle in days 23 and 37 are shown in Table 7. On day 23, no significant differences were obtained with regard to the pH values and WHC among experimental treatments. However, the pH$_u$ of the thigh muscle in the group fed with Cr-met was higher than non-stressed NC group on day 37 ($p = .012$). The percentage of WHC was not affected by exogenous DEX or by treatments on day 37. The

---

**Table 5.** Effects of Supplemental Nano-Cr, Cr-met and Zn-pro on some blood properties of Japanese quail$^1$ under physiological stress (stress period, 23th day).

| Treatment | NC   | PC   | Nano-Cr | Cr-met | Zn-pro | Pooled SEM | $p$ Value |
|-----------|------|------|---------|--------|--------|------------|-----------|
| WBC$^2$ (10$^3$ cells/mm$^3$) | 4.19$^b$ | 4.06$^a$ | 4.10$^b$ | 4.07$^b$ | 4.11$^a$ | 0.014 | .021 |
| Heterophil (%) | 9.33$^b$ | 13.00$^a$ | 10.00$^a$ | 12.33$^b$ | 9.67$^b$ | 0.380 | .048 |
| Lymphocyte (%) | 86.67$^a$ | 81.33$^b$ | 86.67$^a$ | 80.00$^b$ | 86.33$^a$ | 0.463 | .005 |
| Heterophil: Lymphocyte | 0.11$^c$ | 0.16$^c$ | 0.12$^c$ | 0.15$^a$ | 0.11$^c$ | 0.005 | .003 |
| Monocyte (%) | 4.00 | 5.67 | 4.33 | 7.67 | 4.00 | 0.005 | .277 |
| Haemoglobin (g/dl) | 14.13$^a$ | 10.47$^b$ | 12.10$^a$ | 13.07$^b$ | 11.77$^b$ | 0.312 | .041 |
| Haematocrit (%) | 43.00$^a$ | 32.00$^b$ | 36.67$^a$ | 36.00$^b$ | 36.67$^b$ | 1.090 | .047 |

$^a$–$^c$ Mean values within a row with no common superscript differ significantly ($p < .05$).

NC: negative control (no-DEX, no-additive), PC: positive control (0.6 mg/kg BW DEX, no-additive), Nano-Cr (PC + 800 μg Nano-Cr/kg diet), Zn-pro (PC + 60 mg Zn-pro/kg diet), Cr-met (PC + 800 μg Cr-met/kg diet).

1 Data are means of four observations per diet.

2 White Blood Cell.

**Table 6.** Effects of supplemental Nano-Cr, Cr-met and Zn-pro on some blood properties of Japanese quail$^1$ under physiological stress (post-stress recovery period, 37th day).

| Treatment | NC   | PC   | Nano-Cr | Cr-met | Zn-pro | Pooled SEM | $p$ Value |
|-----------|------|------|---------|--------|--------|------------|-----------|
| WBC$^2$ (10$^3$ cells/mm$^3$) | 4.03 | 3.96 | 3.99 | 4.01 | 4.02 | 0.008 | .224 |
| Heterophil (%) | 33.33$^{abc}$ | 36.67$^{abc}$ | 35.67$^{ab}$ | 42.00$^a$ | 38.33$^c$ | 0.671 | .004 |
| Lymphocyte (%) | 63.00$^a$ | 59.33$^{bc}$ | 59.67$^{bc}$ | 54.67$^c$ | 67.67$^a$ | 0.840 | .021 |
| Heterophil: Lymphocyte | 0.53$^{ac}$ | 0.62$^{bc}$ | 0.60$^{ab}$ | 0.77$^a$ | 0.42$^{c}$ | 0.021 | .008 |
| Monocyte (%) | 3.67 | 4.33 | 4.67 | 3.33 | 4.00 | 0.300 | .772 |
| Haemoglobin (g/dl) | 14.17 | 13.57 | 13.60 | 13.27 | 13.43 | 0.133 | .447 |
| Haematocrit (%) | 43.00$^a$ | 41.33 | 40.50 | 40.33 | 40.00 | 0.410 | .364 |

$^a$–$^c$ Mean values within a row with no common superscript differ significantly ($p < .05$).

NC: negative control (no-DEX, no-additive), PC: positive control (0.6 mg/kg BW DEX, no-additive), Nano-Cr (PC + 800 μg Nano-Cr/kg diet), Zn-pro (PC + 60 mg Zn-pro/kg diet), Cr-met (PC + 800 μg Cr-met/kg diet).

1 Data are means of four observations per diet.

2 White Blood Cell.

**Table 7.** Effects of supplemental Nano-Cr, Cr-met and Zn-pro on meat quality of Japanese quail$^1$ under physiological stress.

| Treatment | NC | PC | Nano-Cr | Cr-met | Zn-pro | Pooled SEM | $p$ Value |
|-----------|----|----|---------|--------|--------|------------|-----------|
| 23 day of age | | | | | | | |
| pH$_0$ | 6.70 | 6.68 | 6.73 | 7.05 | 7.10 | 0.084 | .467 |
| pH$_u$ | 5.98 | 6.38 | 6.74 | 6.39 | 6.07 | 0.133 | .542 |
| ΔpH | 0.72 | 0.30 | 0.01 | 0.07 | 0.03 | 0.120 | .255 |
| WHC$^4$ (%) | 63.01 | 64.33 | 69.9 | 68.83 | 67.46 | 1.070 | .484 |
| 37 day of age | | | | | | | |
| pH$_0$ | 6.57 | 6.76 | 6.43 | 6.17 | 6.86 | 0.054 | .276 |
| pH$_u$ | 6.12$^{a}$ | 6.25$^{ab}$ | 6.34$^{a}$ | 6.58$^{a}$ | 6.50$^{ab}$ | 0.042 | .012 |
| ΔpH | 0.54 | 0.51 | 0.09 | 0.04 | 0.36 | 0.070 | .260 |
| WHC$^4$ (%) | 60.44 | 64.98 | 64.04 | 59.98 | 64.06 | 1.144 | .683 |
| MDA$^5$ (mg/kg of meat) | 0.72$^c$ | 2.50$^a$ | 1.55$^b$ | 2.10$^{ab}$ | 1.61$^{b}$ | 0.092 | .004 |

$^a$–$^c$ Mean values within a row with no common superscript differ significantly ($p < .05$).

NC: negative control (no-DEX, no-additive); PC: positive control (0.6 mg/kg BW DEX, no-additive), Nano-Cr (PC + 800 μg Nano-Cr/kg diet), Zn-pro (PC + 60 mg Zn-pro/kg diet), Cr-met (PC + 800 μg Cr-met/kg diet).

1 Data are means of four observations per diet.

2 The ultimate pH value of the muscle.

3 Assignments of pH change.

4 Water-Holding Capacity.

5 Malondialdehyde.
concentration of MDA pertaining to the negative control was significantly lower than in other experimental treatments ($p = .004$). Nonetheless, diets that were supplemented with Zn-pro and Nano-Cr reduced MDA ($p = .004$) in the thigh muscle of stressed quails compared to the PC group at the age of 37 days.

### Carcase characteristics

The results of carcase characteristics at 23 and 37 days of age are presented in Table 8. Our results showed that physiological stress can lead to an increase in the relative liver weight during the stress period and also an increase in the relative gut weight during the recovery period in the PC group as compared to non-stressed quails ($p = .004$) (Table 8). The relative weights of liver and heart of the birds during stress period (on day 23) were unaffected significantly by the dietary treatments. Relative weights of heart, liver (on day 37) and carcase yield were not affected by the dietary supplements.

### Discussion

Results in the present study indicated that physiological stress reduced feed consumption, live body weight, and feed efficiency in quails that experienced stress. Dexamethasone and other glucocorticoid analogs usually suppress or inhibit the growth of poultry. In line with the results of this study, Li et al. (2009) reported that injecting DEX to broilers at different doses can significantly decrease the final body weight. This phenomenon indicated that a lower feed consumption in quail chicks challenged by glucocorticoids was a reason for the retarded growth. Also, our study confirms that the actual status of stress can be successfully induced by the oral administration of DEX. Here, it was shown that the growth rate is strongly influenced by physiological stress in quail chicks during the stress period. Over the recovery period, however, weight gain increased among the stressed birds in comparison to the negative control group. This indicates the compensatory occurrence of growth in stressed birds at the end of the recovery period. Relevant to this context, it is known that physiological stress causes an increase in the catabolism of muscle proteins, thereby reducing the BW (Puvadolpirod and Thaxton 2000b). Generally, Zn and Cr are known as the main anti-stress micronutrients (Sahin et al. 2005; Attia, Abd Al-Hamid, Zeweil, et al. 2013; Attia, Abd Al-Hamid, ElKomy, et al. 2013; Attia et al. 2019). BW and weight gain of quails were not significantly affected by the dietary supplements in the experiments. This is because the stress intensity in our study might have been substantially higher than in previous trials. In fact, contrary to this study, previous researchers reported that the organic source of Zn in the diet of quails can increase body weight and feed efficiency, thereby improving the feed intake and carcase quality under heat stress (Sahin et al. 2005, 2006; Attia et al. 2019). However, this study suggests that the feed intake of quails in the Zn-pro treatment during the stress period was higher than in other stressed groups. This is similar to the results reported by Sahin et al. (2005). Zinc is involved in the proper function of sex and growth hormones, glucagon, insulin (Sahin et al. 2005) and many other reactions (Attia, Abd Al-Hamid, Zeweil, et al. 2013; Attia, Abd Al-Hamid, ElKomy, et al. 2013). Due to the fact that Zn has a wide array of functions, it can affect the digestive system because it has a protective role in the pancreatic tissue and safeguards it against oxidative damages. Therefore, Zn may help the pancreas to function properly, by enabling the secretions of enzymes involved in the digestive system and ultimately improving the digestibility of nutrients, thereby prompting the birds to consume more feed (Saleh et al. 2018). To provide further support for the constructive role of Zn under stress conditions.

| Treatment | NC | PC | Nano-Cr | Cr-met | Zn-pro | Pooled SEM | $p$ Value |
|-----------|----|----|---------|--------|--------|------------|----------|
| 23 day of age | | | | | | | |
| Relative liver weight (%) | 2.82b | 4.13a | 3.64ab | 3.36ab | 3.56ab | 0.122 | .004 |
| Relative heart weight (%) | 0.93 | 1.13 | 0.96 | 1.25 | 1.09 | 0.051 | .468 |
| 37 day of age | | | | | | | |
| Hot carcase weight (g) | 126.93a | 93.83b | 91.93b | 95.73b | 94.60b | 3.123 | .033 |
| Carcase yield (%) | 0.57 | 0.59 | 0.60 | 0.62 | 0.60 | 0.006 | .357 |
| Relative gut weight (%) | 10.68a | 15.09b | 12.73ab | 12.46ab | 12.05ab | 0.422 | <.001 |
| Relative liver weight (%) | 2.20 | 2.67 | 2.53 | 2.36 | 2.34 | 0.084 | .559 |
| Relative heart weight (%) | 0.95 | 0.96 | 0.84 | 0.79 | 0.82 | 0.021 | .274 |

(a,b)Mean values within a row with no common superscript differ significantly ($p < .05$). NC: negative control (no-DEX, no-additive); PC: positive control (0.6 mg/kg BW DEX, no-additive), Nano-Cr (PC + 800 µg Nano-Cr/kg diet), Zn-pro (PC + 60 mg Zn-pro/kg diet), Cr-met (PC + 800 µg Cr-met/kg diet).

1Data are means of four observations per diet.
conditions, Sahin et al. (2005) reported that Zinc Picolinate (ZnPic) can be used as an inorganic source of Zn which reduces the adverse impacts of oxidative stress induced by heat stress in quails. The same report also declared that the supplementation of ZnPic improves carcass weight.

Chromium is recognised as a glucose tolerance factor (GTF) which helps cellular binding and augments the action of insulin by regulating the metabolism of proteins, fat and carbohydrates (Uyanik et al. 2002). In addition, previous studies demonstrated that diets which are supplemented with Cr can improve the detrimental effects of heat stress and could boost the performance of broiler chickens under heat stress (Sahin et al. 2005, 2010; Ghazi et al. 2012). The results of our study demonstrated reductions in the ratios of gain to metabolisable energy intake and gain to protein intake among stressed birds. This means an improvement in energy expenditure. The increased energy expenditure could be triggered by very high levels of DEX circulating in the bloodstream. This is probably because the formation of reactive oxygen species is a consequence of the increase in lipid peroxidation (Lin et al. 2004a,b). In line with these results, Lin et al. (2006) reported that the administration of corticosterone to broiler chickens can increase their energy expenditure due to alterations in metabolism.

In the present study, the total count of white blood cell, the percentage of lymphocytes, haemoglobin concentration, and haematocrit percentage were depressed by DEX during the stress period. In agreement with these results, Adedapo et al. (2004) reported anaemia as a result of DEX administration in broiler chicks, whereby the Hb concentration declined in that study. In contrast to our results, Aengwanich (2007) reported that injecting DEX into broiler chicks caused an increase in WBC, Hb, and Hct. Generally known, corticosterone causes heterophilia (i.e. the ability to react immunologically with materials of another species). Heterophilia occurs when the release of heterophils increases from the bone marrow reserves, and as they find their way through to the circulation. This response is called the non-specific immune response which is an important physiological response to stressors (Jain 1993). Jain (1993) also explained that dexamethasone causes lymphocytopenia which is attributed to lympholysis in the bloodstream and could also be a result of lymphoid tissue atrophy. Accordingly, the count of lymphocytes could decrease in the blood circulation. In agreement with our results, Sirirat et al. (2012) found that Nano-Cr supplements can increase the count of lymphocytes in broiler chicks. It is reported that Nano-Cr can enhance the lymphoproliferative response in rats, which also means that Nano-Cr can boost lymphocyte counts (Zha et al. 2009a). In the present study, Nano-Cr and Zn-pro supplements increased lymphocyte counts in Japanese quails under stress and also post-stress period. Zinc is known to enhance the production of antibodies, interleukin-2 and interferon, thereby contributing to the rise in macrophages and increasing the counts of thymocytes, peripheral T-cells (Sunder et al. 2008) and lymphocytes. Furthermore, Sunder et al. (2008) found that broilers fed with Zn supplements had heavier lymphoid organs such as spleen and bursa in comparison with the control group. Monocytes percentage was uninfluenced by the physiological stress, neither in the stress period nor by the end of the recovery period. Therefore, immune responses were not observed to be affected by physiological stress. Here, our results of the stress index (H: L) indicated that Zn-pro reduced the H: L ratio of birds during the stress period. In agreement with this finding, Sunder et al. (2008) reported that Zn supplementation reduced the H: L ratio compared to the control group. Consequently, it can be said that Zn-pro is more efficient in moderating the stress in recovery period, compared to the actions of Cr-met or Nano-Cr in this trial.

Our findings revealed that in quails exposed to DEX, the pH values were observed to increase linearly at 23 and 37 days of age than NC group. Stress accelerates the glycogen depletion, and then increases the changes to the pH values of meat. It would ultimately cause glycogen deficiency among stressed birds in which the deficiency of muscle glycogen would result in lower rates of glycolysis in muscles after death, and a higher pH value would ensue (Ali et al. 2008). The concentrations of MDA in the meat of stressed quails were notably higher than in non-stressed quails due to lipid peroxidation under stressful conditions. Secretion of catecholamines and corticosterone in birds due to physiological stress can lead to lipid peroxidation in the cell membrane which explains a part of our results, indicating that the exogenous administration of DEX for a long time (more than 5 days) can enhance lipid peroxidation in the skeletal muscles of broiler chicks (Gao et al. 2010). Zinc-proteinate and Nano-Cr significantly reduced MDA values in the thigh muscle of stressed quails. This could mean that Zn-pro and Cr – in its nanoparticle form – have antioxidant properties. Zinc has been defined as an important antioxidant, and as a micronutrient, that protects the cell membrane against oxidative damage, while it plays a key role in the suppression of free radicals and
could induce the production of metallothionein as an agent that scavenges free radicals (Sahin et al. 2006b). The current results showed that during stress period, the improvement in FI by Zn-pro has a relation to hematological stress index (lower H: L ratio). Although pHu was not significantly lower than in dietary treatments, there was a numerical decrease in this parameter in quails digested Zn-pro during the stress period. Muscles pH can play an essential role in microbial spoilage rate and also low pH of meat can increase shelf-life. It seems that, in stressed birds that are highly susceptible to an increase in their ultimate pH of meat, an organic source of Zn might prevent to rise in pHu due to the stress conditions. Therefore, Zn dietary supplementation in quails during period of severe stress probably due to its effect on reducing the stress or inflammation index (H: L ratio) may improve feed intake and reduce the pH of meat after slaughtering.

It is obviously seen that Cr mimics the role of GTF which potentiates the biological action of insulin (as the insulin cofactor) by enhancing the sensitivity of receptors to insulin. It has been well recognised that the metabolism of insulin influences lipid peroxidation, while stimulating the activity of insulin due to the reduction in some stress hormones (like epinephrine) which inhibit the breakdown of lipids, thereby suggesting that Cr may function as an antioxidant (Preuss et al. 1997). It is reported that Cr supplements can increase the serum concentration of antioxidant vitamins such as vitamins C and E (Sahin et al. 2003). As a result, malondialdehyde concentrations in the serum of broiler chicks in some reports have been observed to be low (Sahin et al. 2003). Due to the small size and high surface area of nanoparticles, and their comparison with micro scales, the experimented compounds used in this study are more bioavailable and can be absorbed more efficiently by the digestive system. Accordingly, Wang et al. (2014) reported that Cr nanoparticles have useful effects on the meat and carcass characteristics of pork, including the reduction in drip loss.

This study revealed that some carcass traits of quails such as carcass yield and the relative weight of heart were not influenced by the induction of physiological stress. However, the increase in the liver weight, as a percentage of body mass, at the age of 23 days is probably related to the hypertrophy of the liver in quail chicks exposed to DEX (Odihambo Mumma et al. 2006). In addition to this, chronic stress in this study led to a dramatic decrease in BW which could reduce the relative weight of internal organs. Stress, as induced by the administration of corticosterone or its analogs, has significantly increased the liver weight in different studies (Malheiros et al. 2003; Cai et al. 2009). The increase in the absolute weight of the liver and the simultaneous decrease in body weight (relative weight of the liver) indicate the hypertrophy of the liver due to a concomitant accumulation of lipids (Lin et al. 2006). Birds challenged with DEX showed an increase in hepatic lipogenesis which is a form of augmented fat deposition in the liver (Cai et al. 2009). Puvadolpirod and Thaxton, (2000a) also reported that the continuous administration of ACTH could prompt higher lipid accumulation in the livers of broiler chicks. On the other hand, Odihambo Mumma et al. (2006) indicated that an increase in the liver weight of laying hens, as caused by the ACTH treatment, may be related to higher levels of moisture, protein, carbohydrate and fat in the liver, along with more ash. Therefore, the increase in lipid accumulation could explain heavier weights of the liver in quails during the stress period. As mentioned above, physiological stress leads to the suppression of skeletal growth, along with an increase in muscle catabolism in birds and the reduction in their BW. Under stress conditions, the gut growth is maintained by utilising nutrients, but at the expense of limiting the skeletal growth. As a result, the relative weights of the guts increased in this study, which is in line with previous results reported by Hu et al. (2010). Nonetheless, there could be another explanation: physiological stress impairs the intestinal barrier, which is associated by inflammatory reactions because of damages occurring to the mucosa barrier. This would cause the entrance of unwanted substances such as food antigens, lipopolysaccharides, bile and hydrolytic enzymes into the internal environment of the bowel, and therefore the stress causes dysfunctions of the intestinal barrier. As a result, heightened levels of intestinal inflammation, permeability, and the relative weight of bowel could be observed in stressed birds (Lambert 2009). The increase in the relative weight of the heart at the age of 23 days in stressed birds is comparable to that of the non-stressed ones, and this difference is probably related to a rise in the pulse rate and blood pressure followed by an increase in the activity of the circulatory system under stressful conditions (Siegel 1995).

**Conclusions**

In conclusion, the results of the present study suggest that Zn-pro supplementation (60 mg/kg) can improve the feed intake, meat oxidative stability and reduce
stress index (heterophil: lymphocyte ratio) of quails under physiological stress induced by exogenous DEX at dose 0.6 mg/kg BW.

Further research needs to be conducted to provide a better understanding of the beneficial effects of Zn-pro, Nano-Cr, and Cr-met in quails to alleviate the adverse effects of stress.

Acknowledgements

The authors would like to acknowledge SANA® group Co. (Tehran, Iran) for donating chromium methionine.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The study was funded and supported by the University of Tehran.

ORCID

Seyed Davood Sharifi http://orcid.org/0000-0002-4129-7600

References

Adedapo AA, Saba AB, Dina OA, Oladejo GMA. 2004. Effects of dexamethasone on the infectivity of Trypanosoma vivax and serum biochemistry change in Nigerian domestic chickens. Veterinarni arhiv Trop Anim Sci. 74(5):371–382.

Aengwanich W. 2007. Effects of dexamethasone on physiological changes and productive performance in broilers. Asian J Anim Vet Adv. 32(2):157–161.

Aengwanich W, Chinarsri O. 2003. Effect of dexamethasone on differential white blood cell counts and heterophil/lymphocyte ratio in Japanese quails (Coturnix coturnix japonica). Songklanakarin J Sci and Tech. 25:183–189.

Ali M, Kang GH, Joo ST. 2008. A review: Influences of pre-slaughter stress on poultry meat quality. Asian Australas J Anim Sci. 21(6):912–916.

Attia YA, Abd-Al-Hamid AE, ElKomy A, Shawky OM. 2013. Responses of productive, physiological and immunological traits of growing Fayoumi males subjected to stress to vitamin C and/or E and organic Zinc supplementation. J Agric Envi Sci Dam Univ. 12(1):48–75.

Attia YA, Addeo NF, Al-Hamid A, Bovera F. 2019. Effects of phytase supplementation to diets with or without zinc addition on growth performance and zinc utilization of white pekin ducks. Animals. 9(5):280.

Attia YA, Al-Harthy MA, Sh. Elnaggar A. 2018. Productive, physiological and immunological responses of two broiler strains fed different dietary regimens and exposed to heat stress. Ital J Anim Sci. 17(3):686–697.

Botsoglou NA, Florou-Paneri P, Christaki E, Fletouris DJ, Spais AB. 2002. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. Br Poult Sci. 43(2):223–230.

Cai Y, Song Z, Zhang X, Wang X, Jiao H, Lin H. 2009. Increased de novo lipogenesis in liver contributes to the augmented fat deposition in dexamethasone exposed broiler chickens (Gallus gallus domesticus). Comp Biochem Physiol C Toxicol Pharmacol. 150(2):164–169.

Castellini C, Mugnai CA, Dal Bosco A. 2002. Effect of organic production system on broiler carcass and meat quality. Meat Sci. 60(3):219–225.

Davis AK, Maney DL, Maerz JC. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct Ecol. 22(5):760–772.

Gangadoo S, Dinev I, Chapman J, Hughes RJ, Van TT, Moore RJ, Stanley D. 2018. Selenium nanoparticles in poultry feed modify gut microbiota and increase abundance of Faecalibacterium prausnitzii. Appl Microbiol Biotechnol. 102(3):1455–1466.

Gao J, Lin H, Wang XJ, Song ZG, Jiao HC. 2010. Vitamin E supplementation alleviates the oxidative stress induced by dexamethasone treatment and improves meat quality in broiler chickens. Poult Sci. 89(2):318–327.

Ghazi SH, Habibian M, Moeini MM, Abdolmohammadi AR. 2012. Effects of different levels of organic and inorganic chromium on growth performance and immunocompetence of broilers under heat stress. Biol Trace Elem Res. 146(3):309–317.

Gultepe EE, Uyarlar C, Bayram I. 2018. Supplementation of cr methionine during dry period of dairy cows and its effect on some production and biochemical parameters during early lactation and on immunity of their offspring. Biol Trace Elem Res. 186(1):143–153.

Hu XF, Guo YM, Huang BY, Bun S, Zhang LB, Li JH, Liu D, Long FY, Yang X, Jiao P. 2010. The effect of glucagon-like peptide 2 injection on performance, small intestinal morphology, and nutrient transporter expression of stressed broiler chickens. Poult Sci. 89(9):1967–1974.

Jain NC. 1993. Essentials of veterinary hematology. Philadelphia (PA): Lea and Febiger. p. 222–257.

Kececi T, Oğuz H, Kuroğlu V, Demet O. 1998. Effects of polyvinylpolypyrrolidone, synthetic zeolite and bentonite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. Br Poult Sci. 39(3):452–458.
Wang MQ, Wang C, Du YJ, Li H, Tao WJ, Ye SS, He YD, Chen SY. 2014. Effects of chromium-loaded chitosan nanoparticles on growth, carcass characteristics, pork quality, and lipid metabolism in finishing pigs. Livestock Sci. 161: 123–129.

Zha LY, Zeng JW, Chu XW, Mao LM, Luo HJ. 2009a. Efficacy of trivalent chromium on growth performance, carcass characteristics and tissue chromium in heat-stressed broiler chicks. J Sci Food Agric. 89(10): 1782–1786.

Zha L, Zeng J, Sun S, Deng H, Luo H, Li W. 2009b. Chromium(III) nanoparticles affect hormone and immune responses in heat-stressed rats. Biol Trace Elem Res. 129(1–3):157–169.