Effect of dietary saffron (*Crocus sativus*) petal extract on growth performance, blood biochemical indices, antioxidant balance, and immune responses of broiler chickens reared under heat stress conditions

Seyyed Javad Hosseini-Vashan* and Ali Hossein Piray*

*Department of Animal Science, Faculty of Agriculture, University of Birjand, Birjand, Iran; **Department of Animal Science, College of Agriculture and Natural Resources, Razi University, Kermanshah, Iran

**ABSTRACT**

To evaluate the alleviative effects of saffron (*Crocus sativus*) petal extract (SPE) on growth performance, blood biochemical indices, antioxidant status, and immunity of heat-stressed broilers, 250 newly hatched male broiler chickens were randomly allocated to control diet (basal diet, two groups), or control diet supplemented with 300, 500, or 700 mg SPE/kg diet, with five replicates and 10 birds each. The broiler chickens, except one of the control groups, were exposed to heat stress (HS, 37 ± 1°C, 55% relative humidity) for 6 h/day from day 25 to 42. Heat stress reduced body weight gain (BWG), feed intake (FI), and abdominal fat percentage, and increased feed conversion ratio (FCR, *p* < .05). It also caused decreases in the antibody production against sheep red blood cells (SRBC) and Newcastle disease virus (NDV) and an increase in the heterophil: lymphocyte (H: L) ratio. However, SPE linearly increased the FI during the entire experimental period (*p* = .024) and linearly improved the BWG and FCR during the finisher and entire experimental periods (*p* < .05). The abdominal fat percentage decreased linearly with increasing dietary SPE levels (*p* < .005). Dietary SPE also caused significant reductions in the plasma concentration of cholesterol (L, *p* < .001), uric acid (L, *p* < .001), and MDA (L, *p* < .001; Q, *p* < .001) and enzyme activity of LDH (L, *p* = .002; Q, *p* = .015) and AST (L, *p* = .002; Q, *p* = .019), whereas enhanced the total protein concentration (L, *p* = .006), SOD and GPx (L, *p* < .001; Q, *p* = .028) activities, and TAC content (L, *p* < .001). Meanwhile, supplementation with dietary SPE enhanced the bursa percentage (*p* = .05) and improved the primary and secondary antibody production titres against SRBC and antibody response to NDV at 24 and 24 days (*p* < .01). The results indicate that SPE is an effective additive for broiler chickens under heat stress conditions. According to estimates of regression models, the optimum amount of extract recommended is 689.5 mg/kg.

**HIGHLIGHTS**

- Saffron petal extract (SPE) enhanced the growth performance of heat-stressed broiler chickens.
- Dietary SPE improved the liver function in broilers reared in heat stress conditions.
- Supplementation of SPE improved the antioxidant status and immune responses in heat-stressed broiler chickens.

**Abbreviations:** AST: aspartate aminotransferase; BWG: body weight gain; FCR: feed conversion ratio; FI: feed intake; GPx: glutathione peroxidase; HDL: high-density lipoprotein; H: L: heterophil: lymphocyte; HS: heat stress; LDH: lactate dehydrogenase; MDA: malondialdehyde; NDV: Newcastle disease virus; SOD: superoxide dismutase; SPE: saffron petal extract; SRBC: sheep red blood cells; TAC: total antioxidant capacity

**Introduction**

Heat stress (HS), as one of the main stressors for the poultry industry in the tropical, subtropical, and arid regions, significantly reduces production efficiency in these areas. Increasing evidence indicates that HS has adverse effects on various aspects of poultry biology. Previous reports showed that HS exposure impaired growth performance, induced tissue damage,
weakened the immune system, and reduced the quantity and quality of meat produced (Khan et al. 2011, 2012; Safdari-Rostamabad et al. 2017; Sharifian et al. 2019; Hosseini-Vashan et al. 2016, 2020). Heat stress also increases the production of free radicals and reduces the antioxidant defense, and leads to oxidative stress (Hosseini-Vashan et al. 2016; Safdari-Rostamabad et al. 2017). Free radicals are highly reactive and attack all biomolecules, including lipids, proteins, and nucleic acids, resulting in mitochondrial dysfunction and cellular apoptosis. Oxidative stress is associated with the harmful effects of HS. Currently, the poultry industry uses medicinal plants and their derived products to reduce and/or counteract the effects of HS (Hosseini-Vashan et al. 2016; Sharifian et al. 2019).

In heat stress condition, supplementation of vitamin E and ascorbic acid into bird’s diet improve the growth performance, feed efficiency, nutrient digestibility, immune response and antioxidant balance (Khan et al. 2011, 2012). Saffron (Crocus sativus), a plant of the Iris family, is primarily used as food flavour and colour. Iran is the largest producer of saffron in the world, producing 404.5 tons of saffron in 2018 (Ahmadi et al. 2019). Producing one kilogram of dried saffron produces a lot of byproducts, including saffron petals (350 kg), leaves (1500 kg), and corms, which are discarded without being used (Lahmass et al. 2018). Saffron petals are dried in the shade, powdered, then concentrated under vacuum using a rotary evaporator. It was stored at 4°C until use (Goli et al. 2012).

Table 1. The bioactive component of saffron petal extract (SPE).

| Item          | Total phenol | Flavonoid | Anthocyanin | FRAP<sup>a</sup> |
|---------------|--------------|-----------|-------------|------------------|
|               | (mg / 100g)  | (mg / 100g)| (mg / 100g) | 5.3 mmol         |
| Flavonoid     | 153          |           |             |                  |
| Anthocyanin   | 114          |           |             |                  |
| Total phenol  | 1450         |           |             |                  |

<sup>a</sup>FRAP: The ferric reducing ability of plasma. Values are mean of 5 samples.

The saffron was manually removed from saffron petals. The saffron petals were dried in the shade, powdered using a grinding mill, and then macerated for 72 h in ethanol (98%, v/v) at room temperature. The extract was filtered, then concentrated under vacuum using a rotary evaporator. It was stored at 4°C until use (Goli et al. 2012). To assess the polyphenols content of homogenate extract, the saffron extract was centrifuged (10 min, 25°C, 3000 g), and the supernatant was collected. The total anthocyanins substance was determined by a spectrophotometric differential pH method. The content of total anthocyanins in saffron extract was expressed as mg of cyanidin 3-glucoside/100 g DM (Connor et al. 2002). The Folin-Ciocalteu reagent method was used to determine the extractable polyphenols (Montreau 1972). The total polyphenol content was determined at 765 nm absorbance and expressed as mg of gallic acid (GA)/g DM, using a calibration curve (Montreau 1972). The polyphenols content of the SPE were presented in Table 1.

Animals and diets

All experimental procedures involving the use of animals were approved by the Birjand University Institutional Animal Care and Use Committee. A total of 250 newly hatched male broiler chickens (Ross 308) with similar body weights (40 ± 2 g) were purchased from a local commercial hatchery. They were randomly assigned to five groups of 50 broiler chickens, each with five replicate floor pens and fed with basal diet (two control groups), either the basal diet supplemented with 300, 500, or 700 mg SPE/kg diet from 1 to 42 days of age. The basal diets consisting of starter, grower, and finisher diets were formulated to meet or exceed the nutrient requirements (Aviagen 2014,
Table 2) and fed from 1 to 10, 11 to 24, and 25 to 42 days, respectively. All broiler chickens were reared under standard management conditions until day 24. After then, one of the control groups was kept in a temperature-controlled room at 21 ± 2°C for 24 h/day (thermoneutral, TN, group). Other remaining groups were raised in another temperature-controlled room and subjected to 14 h of 21°C, 2 h of 21 to 34°C, 6 h of 37 ± 1°C from 10:30 h to 16:30 h, and 2 h of 34 to 21°C from day 25 to 42. The average relative humidity of the rooms was 55%. Feed in mash form and fresh-water were provided ad libitum, and continuous light (22 h light: 2 h dark) was applied throughout the experimental period.

**Growth performance and carcase characteristics**

Feed intake and body weight were recorded for each pen at 10, 24, and 42 days, then body weight gain (BWG) and feed conversion ratio (FCR) were calculated for each pen.

At the end of the study, two birds were selected randomly from each replicate (10 broiler chickens per treatment), weighed, and slaughtered. The weight of carcase, breast, thigh and drumstick, abdominal fat, liver, pancreas, gallbladder, heart, gizzard, bursa of Fabricius, and spleen was measured individually and expressed as a percentage of live weight.

**Blood metabolites and antioxidant and immune responses**

On day 42, two broiler chickens per replicate were selected randomly, and blood samples were taken from the brachial vein 4 h after feed withdrawal. Blood samples were centrifuged (3,000 g for 10 min), and collected plasma samples were stored at −20°C until analysis. The plasma concentrations of total protein (TP), triglyceride (TG), cholesterol, high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) and the enzyme activities of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were determined using commercial kits (Pars Azmoun Co., Tehran, Iran). At the end of the study (42 d), plasma lipid peroxidation was measured as thiobarbituric acid-reactive substances according to Yoshioka et al. (1979). Glutathione peroxidase (GPx) and superoxide dismutase (SOD) enzyme activities were measured in erythrocyte hemolysates following the manufacturer instructions (Ransel and Ransod test kits, Randox Laboratories Ltd, UK.).

To assess the effect of HS and SPE on humoral immunity, three chickens per replicate pen were randomly selected and marked with coloured, numbered identification tags. Two birds were randomly inoculated intravenously with 1 mL of 10% sheep red blood cells (SRBC) diluted in phosphate-buffered saline (PBS), and the third was injected with PBS at 18 and 35 days. The broiler chickens were immunised against Newcastle disease virus (NDV) at 8, 18, and 28 days of age. On day 24 and 42, blood samples were collected from the birds, and titres of the total, immunoglobin (Ig) G and IgM antibodies against SRBC and antibody production response against NDV were measured using microhemagglutination inhibition test (Hosseini-Vashan et al. 2016).

**Statistical analysis**

The assumptions of ANOVA were checked using the Shapiro–Wilks test for normality and the Levene test.
for the homogeneity of the variances. To determine the effects of HS, the means of TN control and HS control were compared using t-test if the parametric conditions existed; otherwise, the nonparametric Mann–Whitney U test was used. Linear and quadratic effects of the SPE levels in the HS conditions were also evaluated using SPSS (18.0). Broken-line (Robbins et al. 2006) and polynomial regression analyses were applied to determine the optimal level of dietary SPE (SAS 9.1). Furthermore, principal component analysis (PCA) was performed to find associations between overall feed efficiency (BWG/FI) and some blood indices of broiler chickens fed increasing levels of SPE in the HS conditions, and the varimax rotation method was employed in the rotation of the factor matrix to improve the interpretability of the principal components (SPSS 18.0). We considered $p \leq .05$ as significant, and $.05 < p < .10$ as trend.

**Results**

Dietary supplementation with SPE did not significantly affect growth performance indices before the onset of HS during the starter and grower periods. However, supplement tended to increase BWG during the starter ($p = .097$) and grower ($p = .052$) periods (Table 3). The results also showed that HS reduced the FI and BWG, and worsened the FCR during the finisher and entire experimental period ($p < .05$). However, SPE supplementation linearly increased the FI during the whole experimental period ($p = .024$), and linearly improved the BWG and FCR during the finisher and whole experimental periods ($p < .05$, Table 4).

Heat stress caused a significant reduction in the abdominal fat percentage ($p = .023$) without affecting the carcass, breast, thigh and drumstick, liver, gallbladder, heart, and pancreas percentages. Saffron petal extract supplementation augmented the decreasing effect of HS on the abdominal fat and further reduced it (L, $p = .009$). It had no impact on the other carcass traits (Table 5).

Exposing to HS resulted in a significant decrease in the plasma TP ($p = .044$) concentration, enzyme activity of GPx ($p = .029$) and SOD ($p = .006$), and TAC content ($p = .029$), while increased the plasma concentration of HDL ($p = .034$), uric acid ($p = .029$), and MDA ($p = .029$) and enzyme activity of LDH ($p = .005$) and AST ($p = .002$). Other plasma

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**Table 3.** Effect of saffron petal extract (SPE) on growth performance of broiler chickens from 1 to 24 d of age (prior to the onset of heat stress).

| Item            | Control | 300 | 500 | 700 | SEM ** | Contrasts p-value* |
|-----------------|---------|-----|-----|-----|--------|--------------------|
| 'Starter' phase (d 1–10) |         |     |     |     |        |                    |
| BWG, g          | 170.5   | 181.4 | 178.8 | 184.7 | 5.17   | 0.097 0.648       |
| FI, g           | 224.7   | 222.3 | 228.3 | 233.3 | 4.19   | 0.106 0.409       |
| FCR, g/g        | 1.32    | 1.23  | 1.28  | 1.27  | 0.052  | 0.647 0.488       |
| 'Grower' phase (d 11–24) |       |     |     |     |        |                    |
| BWG, g          | 633.3   | 781.4 | 681.2 | 735.3 | 22.27  | 0.052 0.061       |
| FI, g           | 966.8   | 967.2 | 1020.6 | 972.1 | 21.79  | 0.051 0.317       |
| FCR, g/g        | 1.54    | 1.24  | 1.50  | 1.33  | 0.040  | 0.096 0.243       |

Values are means, $n = 10$ replicates for the control diet and $n = 5$ replicates for the other treatments with 10 chicks per pen. * BWG, Body weight gain; FI, Feed intake; FCR, Feed conversion ratio; **SEM, standard error of the mean.

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**Table 4.** Effects of heat stress (HS) and saffron petal extract (SPE) on broiler chickens growth performance.

| Item            | TN ** | HS | HS + 300SPE | HS + 500SPE | HS + 700SPE | SEM *** | TN vs HS | p-value |
|-----------------|-------|----|------------|------------|------------|--------|---------|---------|
| 'Finisher' phase (d 25 to 42) |       |     |          |            |            |        |         |         |
| BWG, g          | 1437.0 | 1020.5 | 1140.7 | 1182.3 | 1177.3 | 43.62  | 0.000  | 0.037  | 0.223  |
| FI, g           | 2968   | 2555.0 | 2723.5 | 2684.0 | 2650.9 | 45.70  | 0.000  | 0.072  | 0.092  |
| FCR, g/g        | 2.07   | 2.51  | 2.42    | 2.27    | 2.26    | 0.082  | 0.000  | 0.050  | 0.714  |
| Whole phase (d 1 to 42) |       |     |          |            |            |        |         |         |
| BWG, g          | 2277.7 | 1826.1 | 2103.4 | 2042.3 | 2097.3 | 39.92  | 0.029  | 0.000  | 0.069  |
| FI, g           | 4168.2 | 3742.1 | 3912.9 | 3932.9 | 3856.3 | 47.46  | 0.000  | 0.024  | 0.053  |
| FCR, g/g        | 1.83   | 2.01  | 1.84    | 1.90    | 1.81    | 0.034  | 0.000  | 0.008  | 0.252  |

Data are the mean of five replicates with 10 birds each.

*BWG, Body weight gain; FI, Feed intake; FCR, Feed conversion ratio; **TN, Thermoneutral group (21 ± 1 °C for 24 h) with basal diet; HS, Broilers were fed basal diet and subjected to heat stress (37 ± 1 °C) for 6 h / d from d 25 to 42 of age; HS + 300SPE, HS + 500SPE, and HS + 700SPE = Broilers were fed basal diet supplemented with 300, 500, and 700 mg SPE / kg from 1 to 42 d and subjected to heat stress (37 ± 1 °C) for 6 h / d from d 25 to 42 of age; ***SEM, standard error of the mean.

**Table 4.** Effects of heat stress (HS) and saffron petal extract (SPE) on broiler chickens growth performance.

| Contrast p-value* |
|-------------------|------------------|------------------|
| Linear            | Quadratic        |
| 0.097 0.648       | 0.106 0.409      |
| 0.647 0.488       |                  |

*Linear and quadratic effects of saffron petal extract.
Table 5. Effects of heat stress (HS) and saffron petal extract on carcase characteristics (% of live weight) of broiler chickens at 42 d.

| Item                      | TN** | HS  | HS + 20GP | HS + 40GP | HS + 60GP | SEM ** | TN vs HT | Linear* | Quadratic |
|---------------------------|------|-----|----------|----------|----------|-------|---------|---------|----------|
| Eviscerated yield         | 61.8 | 62.3| 62.8     | 62.1     | 63.5     | 0.52  | 0.475   | 0.240   | 0.503    |
| Breast                    | 21.4 | 22.0| 22.6     | 22.6     | 22.3     | 0.42  | 0.335   | 0.678   | 0.367    |
| Thigh and drumstick       | 20.1 | 20.4| 20.2     | 19.7     | 20.9     | 0.35  | 0.274   | 0.554   | 0.097    |
| Liver                     | 2.45 | 2.57| 2.19     | 2.05     | 1.93     | 0.100 | 0.447   | 0.002   | 0.269    |
| Abdominal fat             | 2.62 | 2.10| 1.99     | 1.78     | 1.53     | 0.125 | 0.023   | 0.009   | 0.623    |
| Pancreas                  | 0.211| 0.431|0.211    | 0.200    | 0.212    | 0.0916| 0.114   | 0.167   | 0.279    |
| Gallbladder               | 0.083| 0.091|0.069    | 0.093    | 0.096    | 0.0045| 0.627   | 0.450   | 0.302    |
| Heart                     | 0.620| 0.583|0.600    | 0.560    | 0.545    | 0.0283| 0.515   | 0.281   | 0.612    |
| Bursa                     | 0.152| 0.0128|0.128    | 0.156    | 0.187    | 0.0141| 0.686   | 0.050   | 0.955    |
| Spleen                    | 0.104| 0.102|0.112    | 0.119    | 0.105    | 0.0045| 0.892   | 0.740   | 0.295    |

Data are the mean of five replicates per treatment (2 male chickens per replicate).
*TN, Thermoneutral group (21 ± 1°C for 24 h) with basal diet; HS, Broilers were fed basal diet and subjected to heat stress (37 ± 1°C) for 6 h / d from d 25 to 42 of age; HS + 300SPE, HS + 500SPE, and HS + 700SPE = Broilers were fed basal diet supplemented with 300, 500, and 700 mg SPE / kg from 1 to 42 d and subjected to heat stress (37 ± 1°C) for 6 h / d from d 25 to 42 of age; **SEM, standard error of the mean.
*Linear and quadratic effects of saffron petal extract.

Table 6. Effects of heat stress (HS) and saffron petal extract on blood biochemical indices of broilers at 42 d.

| Item                      | TN** | HS  | HS + 20GP | HS + 40GP | HS + 60GP | SEM ** | TN vs HT | Linear* | Quadratic |
|---------------------------|------|-----|----------|----------|----------|-------|---------|---------|----------|
| Serum lipids, mg/dL       |      |     |          |          |          |       |         |         |          |
| Triglyceride              | 41.1 | 41.8| 53.5     | 54.0     | 64.3     | 9.26  | 0.863   | 0.163   | 0.953    |
| Cholesterol               | 165.0| 162.3|134.6    | 143.8    | 125.9    | 4.16  | 0.582   | 0.000   | 0.315    |
| LDL*                     | 104.4| 106.7|86.1     | 100.3    | 89.9     | 7.84  | 0.771   | 0.373   | 0.570    |
| HDL                      | 44.8 | 56.8| 60.8     | 53.8     | 54.7     | 2.73  | 0.034   | 0.350   | 0.626    |
| Enzyme activities, U/L    |      |     |          |          |          |       |         |         |          |
| AST                      | 162.9| 265.5|179.9    | 204.6    | 188.5    | 10.29 | 0.002   | 0.003   | 0.019    |
| LDH                      | 750.0| 1269.8|810.8   | 999.7    | 863.5    | 51.03 | 0.005   | 0.002   | 0.015    |
| Other blood constituents, mg/dL |     |     |          |          |          |       |         |         |          |
| TP                       | 3.53 | 3.06| 3.61     | 3.49     | 3.73     | 0.115 | 0.044   | 0.006   | 0.234    |
| Uric acid                | 53.8 | 92.7| 81.8     | 73.7     | 66.7     | 1.15  | 0.029   | 0.000   | 0.151    |
| Antioxidant indices       |      |     |          |          |          |       |         |         |          |
| MDA                      | 0.755| 2.66| 1.61     | 1.52     | 1.45     | 0.053 | 0.029   | 0.000   | 0.000    |
| GPx                      | 16.53| 7.57| 9.05     | 9.75     | 9.97     | 0.224 | 0.029   | 0.000   | 0.028    |
| SOD                      | 206.5| 172.3|199.5    | 220.0    | 236.0    | 4.55  | 0.006   | 0.000   | 0.291    |
| TAC                      | 1.478| 0.605|0.713    | 0.630    | 0.944    | 0.0245| 0.029   | 0.000   | 0.905    |

Data are the mean of five replicates per treatment (2 male chickens per replicate).
*LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH, lactate dehydrogenase; TP: total protein; MDA: malondialdehyde; GPx: glutathione peroxidase; SOD: superoxide dismutase; TAC: total antioxidant capacity; **TN: Thermoneutral group (21 ± 1°C for 24 h) with basal diet; HS = Broilers were fed basal diet and subjected to heat stress (37 ± 1°C) for 6 h / d from d 25 to 42 of age; HS + 300SPE, HS + 500SPE, and HS + 700SPE = Broilers were fed basal diet supplemented with 300, 500, and 700 mg SPE / kg from 1 to 42 d and subjected to heat stress (37 ± 1°C) for 6 h / d from d 25 to 42 of age; **SEM, standard error of the mean.
*Linear and quadratic effects of saffron petal extract.

biological indices, including cholesterol, TG, and LDL were not significantly influenced by HS. Supplementation with SPE reduced the plasma concentration of cholesterol (L, p < .001), uric acid (L, p < .001), and MDA (L, p < .001; Q, p < .001) and enzyme activity of LDH (L, p = .002; Q, p = .015) and AST (L, p = .002; Q, p = .019), while increased the concentration of TP (L, p = .006), TAC content (L, p < .001), and enzyme activity of SOD (L, p < .001) and GPx (L, p < .000; Q, p = .028; Table 6).

Heat stress conditions did not influence the relative weight of lymphoid organs, including the bursa and spleen (Table 7). However, it caused decreases in the antibody titre against SRBC and NDV and increased the H:L ratio. Dietary SPE improved the primary and secondary antibody production titres against SRBC, antibody response to NDV at 24 and 24 days (p < .01), and increased the bursa percentage (p = .05).

The quadratic models showed that the optimal BWG and FCR of broiler chickens from day 1 to 42 might be achieved with 557.3 and 667.5 mg SPE/kg diet, respectively. A broken line model indicated that the minimum liver percentage might be obtained with 588.9 mg SPE/kg diet. According to regression models, the optimum levels of dietary SPE for minimising plasma AST, LDH, and MDA levels at day 42 were 511.5, 498.6, and 689.0 mg SPE/kg diet, respectively, whereas the maximum levels of TP, GPx, and SOD might be achieved with 588.9 mg SPE/kg diet. According to regression models, the optimum levels of dietary SPE for minimising plasma AST, LDH, and MDA levels at day 42 were 511.5, 498.6, and 689.0 mg SPE/kg diet, respectively (Table 8).

Two principal component (PC) with eigenvalue greater than 1 emerged. The PC1 and PC2 can explain...
Table 7. Effects of heat stress (HS) and saffron petal extract (SPE) on primary and secondary antibody response in broiler chickens.

| Items                  | TN ** | HS | HS + 300SPE | HS + 500SPE | HS + 700SPE | SEM ** | TN vs HS | Linear* | Quadratic |
|------------------------|-------|----|------------|------------|------------|--------|----------|---------|----------|
| Primary SRBC* titre, log² |     |    |            |            |            |        |          |         |          |
| Total antibody         | 7.2  | 5.4 | 6.6        | 8.0        | 8.0        | 0.32   | 0.004    | 0.001   | 0.083    |
| Ig G                   | 2.0  | 1.6 | 2.2        | 2.6        | 5.6        | 0.23   | 0.421    | 0.001   | 0.001    |
| Ig M                   | 5.0  | 3.8 | 4.4        | 5.4        | 2.4        | 0.23   | 0.008    | 0.008   | 0.001    |
| Secondary SRBC titre   |       |    |            |            |            |        |          |         |          |
| Total antibody         | 6.8  | 5.0 | 6.0        | 7.0        | 7.40       | 0.30   | 0.001    | 0.001   | 0.332    |
| Ig G                   | 4.2  | 3.2 | 4.0        | 4.6        | 5.2        | 0.29   | 0.032    | 0.001   | 0.736    |
| Ig M                   | 2.6  | 1.8 | 2.0        | 2.4        | 2.2        | 0.19   | 0.095    | 0.103   | 0.307    |
| Antibody response to NDV, log² |     |    |            |            |            |        |          |         |          |
| NDV titre at 24 d      | 7.2  | 5.4 | 7.0        | 7.4        | 7.6        | 0.26   | 0.001    | 0.001   | 0.018    |
| NDV titre at 42 d      | 6.4  | 4.6 | 6.0        | 7.0        | 7.4        | 0.28   | 0.001    | 0.001   | 0.096    |
| H : L ratio            | 0.444| 0.536| 0.443     | 0.412      | 0.390      | 0.0127 | 0.008    | 0.001   | 0.020    |

Data are the mean of four replicate per treatment (2 male chickens per replicate).

*SRBC: Sheep red blood cells; Ig: Immunoglobulin; NDV: Newcastle disease virus; H : L: Heterophil : lymphocyte; **TN: Thermoneutral group (21 ± 1°C for 24 h) with basal diet; HS: Broilers were fed basal diet and subjected to heat stress (37 ± 1°C) for 6 h/d from d 25 to 42 of age; HS + 300SPE, HS + 500SPE and HS + 700SPE: Broilers were fed basal diet supplemented with 300, 500, and 700 mg SPE/kg from 1 to 42 d and subjected to heat stress (37 ± 1°C) for 6 h/d from d 25 to 42 of age; **SEM: standard error of the mean.

*Linear and quadratic effects of saffron petal extract

Table 8. Prediction of optimal saffron petal extract (SPE, mg/kg) amounts for broilers under heat stress* based on growth performance, some carcass traits and blood metabolites at d 42.

| Variables                   | Preferred model | R² | SPE |
|-----------------------------|----------------|----|-----|
| BWG (g, day 1–42)           | Quadratic      | 0.87| 557.33|
| FCR (g/g, day 1–42)         | Quadratic      | 0.78| 667.5 |
| Liver (%, day 42)           | One slope, straight broken-line | 0.99 | 588.9 |
| MDA (mol/gHb)               | One slope, broken-line        | 0.99 | 445.2 |
| GPX (μmol/gHb)              | One slope, straight broken-line | 0.99 | 535.3 |
| SOD (μmol/gHb)              | One slope, straight broken-line | 0.99 | 674.0 |
| Plasma AST (mg/dL)          | Quadratic      | 0.83| 511.5 |
| Plasma LDH (mg/dL)          | Quadratic      | 0.72| 498.6 |
| Plasma TP (mg/dL)           | Quadratic      | 0.85| 689.5 |

*Heat stress = Broilers were fed basal diet supplemented with 0, 300, 500, and 700 mg SPE/kg diet from 1 to 42 d and subjected to heat stress (37 ± 1°C) for 6 h/d from d 25 to 42 of age.

Discussion

Supplementation with SPE did not affect the growth performance indices before the onset of HS. The results also showed the negative effect of HS on the growth performance of broiler chickens. The detrimental impact of HS on the growth performance of broiler chickens was well documented. For example, Safdari-Rostamabad et al. (2017) showed that HS exposure caused a considerable reduction of FI (11%) and BWG (21%), and a significant increase of FCR (10%) during 25–42 days. The negative effect of HS on weight gain might be associated with decreases in the FI and nutrient digestibility, changes in post-absorptive metabolism, hormonal distribution, oxidative stress, and immune suppression (Dai et al. 2011; Safdari-Rostamabad et al. 2017; Orhan et al. 2019). However, dietary SPE supplementation improved the BWG and FCR during the finisher and whole experimental periods. This improvement could be related to the hepatoprotective, antioxidant, and immune-stimulating activities of the extract. The PCA with varimax rotation confirmed these hypotheses (Figure 1). It has been shown that feeding saffron stigma to laying hens had 44.9% and 32.1% of the total variance, respectively. The PC1 has significant positive associations with enzyme activity of GPx and SOD, TAC content, antibody titre against NDV at 42 day, and secondary total antibody response to SRBC, while has significant negative associations with plasma concentration of uric acid and MDA and H/L ratio. Furthermore, LDH, AST, MDA, and liver percentage had strong positive loadings, while feed efficiency (FE) had strong negative loading on the PC2 (Table 9).
no effect on the performance and external egg quality traits (Botsoglou et al. 2005). The discrepancy between this study and previous studies may be due to the difference of experimental conditions such as animal type and temperature. The results showed that neither HS nor SPE had a significant effect on the carcase, breast, thigh, gallbladder, and heart percentages.

The quality of meat impacts both consumers’ acceptance and health. It has been found that the cholesterol concentration in breast and thigh muscles was positively correlated with blood cholesterol concentration (Salma et al. 2007). Following the results, HS increased the plasma HDL concentration and reduced the abdominal fat percentage without significant effect on the plasma TG, cholesterol, and LDL concentrations. Research findings regarding the effects of HS on the blood lipid profile and abdominal fat accumulation are conflicting. For example, Hosseini-Vashan et al. (2016) reported that HS did not affect the plasma lipid profile and abdominal fat percentage. However, HS exposure increased the serum TG, cholesterol, and HDL concentrations (Sahin et al. 2004), while reduced the abdominal fat percentage (Dai et al. 2011; Saifdari-Rostamabad et al. 2017). This study showed that supplementation with SPE reduced the plasma cholesterol concentration in heat-stressed broiler chickens. It has been demonstrated that saffron and its active constituent crocin modulate the blood lipid profile, and exert this effect via several mechanisms, including inhibition of pancreatic lipase activity, antioxidant activity, increasing the level of adiponectin, activation of the peroxisome proliferator-activated receptor α, and modulation of the heat shock proteins concentrations (Razavi and Hosseinzadeh 2017).

High abdominal fat is an undesirable trait, as it reduces customer satisfaction and reduces feed efficiency in broiler chickens (Hosseini-Vashan et al. 2020). Saffron petal extract reduced the abdominal fat percentage, probably through increasing glucose and lipid metabolism and modulating antioxidant and anti-inflammatory responses (Razavi and Hosseinzadeh 2017).

Aspartate aminotransferase activity is commonly used as an indicator of liver injury (Hosseini-Vashan et al. 2016). Liver damage increased plasma AST enzyme activity and decreased protein synthesis in the liver, resulting in a lower concentration of plasma TP.

Figure 1. Loading plot from principle component analysis (PCA) showing interrelationship of feed efficiency, immunity response, antioxidant indices, some blood indices, and liver and bursa percentages of heat-stressed broiler chickens fed saffron petal extract. LDH: Lactate dehydrogenase; AST: Asperatate aminotransferase; TP: Total protein; UA: Uric acid; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; MDA: Malondialdehyde; TAC: Total antioxidant capacity; NDV: Antibody against Newcastle disease virus at day 42; STSRBC: Secondary total antibody against sheep red blood cells; H : L: Heaterophil : Lymphocyte; FE: Overall feed efficiency. Broilers were subjected to heat stress (37 ± 1 °C) for 6 h/d from d 25 to 42 of age.
Moreover, LDH activity is found in all tissues. Serum LDH activity is not tissue-specific; muscle and liver necroses and haemolysis increase its serum enzyme activity (Kreutzer et al. 2008). Heat-stressed controls exhibited higher plasma AST and LDH enzyme activities and a lower TP concentration than TN controls. However, HS had no effect on liver percentage. The results are partially consistent with Safdari-Rostamabad et al. (2017), who reported that exposing broiler chickens to HS increased the liver percentage, elevated AST activity, and lowered plasma TP concentration without affecting the plasma LDH activity. Dietary SPE reduced the AST enzyme activity, whereas increased the TP concentration in plasma. These results indicated the hepatoprotective effect of SPE. The PCA showed that liver percentage was correlated positively with plasma MDA concentration, while correlated negatively with GPx and SOD activities, and TAC content. The hepatoprotective activity of saffron petals against toxic chemicals and chemotherapeutic drug-induced hepatotoxicity was demonstrated. For example, the administration of cisplatin, a chemotherapeutic drug, reduced the antioxidant enzyme activities and increased MDA concentration resulting in liver injury. However, hydro-alcoholic extract of saffron petals reduced the serum MDA and bilirubin concentrations and enzyme activity of AST and ALT, but increased the serum TP and albumin concentrations (Mohajeri and Doustar 2011). However, it was shown that SPE did not change the serum albumin, TP, and creatinine concentrations and also ALT and AST enzyme activities (Hosseini-Vashan and Raei-Moghadam 2019).

The antioxidant system is mainly composed of antioxidant enzymes such as GPx, SOD, and catalase and non-enzymatic antioxidant substances such as vitamins C and E, β-carotene, and uric acid. At the cellular level, heat stress with an effect on mitochondrial function causes oxidative stress. The production rate of radicals is inversely related to the electron transfer rate in the mitochondrial respiratory chain. Chronic heat stress, via reducing the expression of antioxidant enzymes and reducing the body’s antioxidant reserves, reduces the metabolic capacity of mitochondria, which increases the accumulation of oxygen free radicals and disrupts the oxidative balance leading to oxidative stress (Emami et al. 2020). It has been shown that exposing broiler chickens to HS reduced the activity of antioxidant enzymes and the level of non-enzymatic antioxidants, whereas stimulated the production of free radicals (Sahin et al. 2004; Hosseini-Vashan et al. 2016; Safdari-Rostamabad et al. 2017). Saffron petals possess good antioxidant activity, which is mainly due to the presence of carotenoid and flavonoid compounds, notably glycosides of crocin and kaempferol (Zeka et al. 2015). Sánchez-Vioque et al. (2012) assayed the antioxidant property of saffron petal using several in vitro antioxidant methods, including β-carotene/linoleate model system, reducing power, DPPH and nitric oxide radical scavenging, and iron and copper ion chelation. Petal extract extensively inhibited the β-carotene oxidation and exhibited remarkable scavenging nitric oxide radical and Cu^{2+}-chelating activities. Uric acid is the principal end product of the protein metabolism in birds. Heat stress enhances catabolism of muscle proteins and mobilisation of amino acids to facilitate gluconeogenesis and thus eventually results in elevated plasma uric acid levels. It has been shown that HS can cause kidney damage (Khodadadi et al. 2016); therefore, elevated plasma uric acid levels may support this hypothesis. Uric acid contributes significantly to the total antioxidant capacity in stressful conditions (Lin et al. 2004). A significant positive correlation was found between TAC content and plasma uric acid in the current study (R^2 = 0.89; p < .001). The results also showed that HS exposure reduced the activity of GPx and SOD and TAC content, while enhanced the uric acid and MDA concentrations in plasma. However, SPE alleviated these adverse effects. A limited number of studies have evaluated the antioxidant effect of saffron petals on living organisms. For example, Mohaqiq et al. (2020) found that aqueous extract of saffron stigma and petal enhanced the total antioxidant capacity, while reduced the lipid peroxidation in a dose-dependent manner in diet-induced obese rats. Additionally, Alipour et al. (2019) reported that oral administration of SPE enhanced the activity of GPx. Its cutaneous injection caused an increase in the SOD activity and a decrease in the plasma MDA concentration. However, both methods of SPE administration did not change the plasma TAC content. This study also showed that oral administration of SPE increased the SOD activity, but reduced the MDA concentration in the kidney of lambs. It has been reported that saffron stigma supplementation to laying hens diet reared under TN conditions improved oxidative stability of yolk egg (Botsoglou et al. 2005).

Previous studies have shown that heat stress generally weakens the immune system of poultry, which increases the susceptibility of birds to pathogenic agents. For example, HS inhibited the antibody production against Newcastle and infectious bronchitis disease viruses and SRBC injection and delayed the
developmental stages of primary lymphoid organs (Jahanian and Rasouli 2015; Hosseini-Vashan et al. 2020). Reduced feed intake and consequently reduced consumption of essential nutrients, increased corticosterone release from the adrenal glands, stimulation of inflammatory cytokines production, and induction of oxidative stress in lymphoid organs can account for the negative effect of heat stress on the poultry immune system (Jahanian and Rasouli 2015; Zhang et al. 2018). The present study shows the HS conditions impaired immune function in broiler chickens, as evidenced by lower antibody responses to SRBC and NDV and higher H:L ratio. However, SPE significantly alleviated these detrimental effects, and increased the antibody production titres against SRBC and NDV and the bursa percentage. Saffron and its constituents, crocin, safranal, and crocetin, can strengthen the immune system via regulating the antioxidant system, cytokine and immunoglobulin secretion, histamine release, lymphocyte activation, phagocytosis, and cellular co-receptor expression (Boskabady and Farkhondeh 2016). The PCA confirmed the positive association between bursa percentage and humoral immune responses and antioxidant status. Samarghandian et al. (2017) reported that aqueous saffron extract reduced the serum MDA and nitric oxide levels, enhanced the serum glutathione level, catalase and superoxide dismutase activities, and inhibited the expression of inflammatory cytokines, tumour necrosis factor-alpha, and interleukin-6 in the abdominal aorta of streptozotocin-induced diabetic rats. Additionally, SPE at the dose of 75 mg/kg body weight enhanced the serum IgG in SRBC-immunised rats without affecting the lymphocytes, neutrophils, and monocytes proportions, and spleen histology (Babaei et al. 2014).

Conclusions
The present study revealed that HS exposure impaired growth performance, induced hepatic injury, disturbed antioxidant status, and suppressed humoral immune response of broiler chickens. However, dietary SPE alleviated these detrimental effects. The estimates from the regression models showed that the optimal amount of dietary SPE might be 689.5 mg/kg for broiler chickens under HS.

Ethical approval
All procedures were approved by the Animal Care and Use Committee of the University of Birjand, Birjand, Iran.

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

Funding
This work was supported by the University of Birjand under contract number 53534.

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