Arginine supplementation improves reproductive performance, antioxidant status, immunity and maternal antibody transmission in breeder Japanese quail under heat stress conditions

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ABSTRACT
This study was conducted to determine the effects of arginine (Arg) supplementation on reproductive performance, immune response and antioxidant status in breeder quails reared under heat stress (HS). A total of 125 breeder quails were divided into five groups. One group was kept in an environmentally controlled room (22°C) and considered as thermoneutral and four groups were kept at 34°C and fed a basal diet or a basal diet supplemented with Arg concentrations 1.15, 1.30 and 1.45 times the quail requirements per NRC recommendation. HS decreased performance in birds fed basal diet (p < 0.05) compared with thermoneutral group. Higher feed intake and egg production, and improved feed efficiency and Hugh unit variables occurred in Arg supplemented groups (p < 0.05). Birds receiving Arg under HS had higher maternal serum IgG, egg yolk IgY and offspring serum IgG (mg/ml) indicating that the Arg in the diets significantly influenced the immune response of birds. Also, improved in humoral and cell-mediated immune responses occurred in Arg supplemented groups. Quails receiving the Arg supplemented diets exhibited higher (p < 0.05) plasma and liver activity of superoxide dismutase, catalase and glutathione peroxidase as well as lower (p < 0.05) plasma and liver levels of malondialdehyde compared with the HS group fed the basal diet. Our results show that dietary supplementation with Arg could improve the performance of breeder quails under HS by reducing the negative effects of HS, thus could be considered as a nutritional strategy to alleviate the adverse effects of HS.

HIGHLIGHTS
- Heat stress could negatively impact on reproductive performance, antioxidant status, immunity and maternal antibody transmission in breeder quails.
- Arginine can be used as feed additive for the alleviation of the adverse effects caused by HS in breeder quails.
- The Arg requirement determined by NRC (1994) is for laying quails and this may be insufficient for supporting the reproductive performance and hatchability in breeder quails.

Introduction
High ambient temperature is a great concern in all types of poultry operations. Feed intake, feed efficiency (Lili et al. 2020), egg yield (Franco-Jimenez et al. 2007), internal and external egg quality (Manwar et al. 2006), nutrient utilisation (Loeken and Roth 1983), immune (Vaubourdolle et al. 1990) and reproductive performance (Cheng et al. 2015) are adversely affected by severe heat stress (HS). Also, recently the involvement of HS in inducing oxidative stress has received much interest (Mujahid et al. 2006). Oxidative stress is defined as the presence of reactive species (RS) in excess of the available antioxidant capacity of animal cells (Heinzen and Pollack 2003). The effects of HS are possibly due to acceleration in the rate of reactive oxygen species (ROS) formation and/or an increase in ROS reactivity (Bai et al. 2003).

Feeding management practices such as alteration of energy (Mujahid et al. 2009), protein and amino acid supplementation (Corzo et al. 2003), electrolyte supplementation (Ahmad et al. 2005), feeding time (Daghir 2009) and drinker type and height
were formulated to meet nutrient requirements (Johnson et al. 1989). L-arginine (Arg), as an essential amino acid in poultry, plays a decisive role in multiple physiological processes, like growth and feathering, and serves as a precursor of proteins, creatine, polyamines, L-proline, various hormones and nitric oxides (Khajali and Wideman 2010). The main anti-stress effect of arginine is attributed to corticosterone (Adams et al. 1991) and ACTH (Giordano et al. 1996) secretion inhibition by nitric oxide (a metabolite of arginine). Hassanpour et al. (2010) reported that Arg is the only physiological nitrogen donor in the reactions of nitric oxide production. Nitric oxide which is a highly reactive free radical has assumed an important functional role in many physiological processes such as a crucial role in the reproduction system (Oyeyipo et al. 2015). Also, Arg is an immunologic and diets must be adequate in Arg to provide immunity in poultry (D’Amato and Humphrey 2010). It is known that maternal nutrition affects the immunocompetence of offspring (Uni and Ferket 2004). The Arg requirement determined by NRC (1994) is for laying quails and this may be insufficient for supporting the reproductive performance and hatchability in breeder quails. Also, it has been shown that dietary supplementation of Arg above NRC (1994) recommendation enhanced cellular immunity in birds (Youssef et al. 2015). Therefore, dietary supplementation of breeder diets with Arg above the NRC recommendations may increase the transmission of antibodies to offspring in breeders.

Although there are many studies in the literature on Arg supplementation in birds, however, there are no reports in the literature on Arg supplementation in breeder quails under HS conditions. Therefore, the aim of the present study was to evaluate reproductive performance, antioxidant status and immune response, as well as the transfer rate of maternal immunoglobulin’s to offspring in heat-stressed breeder quails, fed extra Arg in their diets as a nutritional strategy in order to counteract the effects of HS.

Materials and methods
Experimental design and diets

Japanese quails were obtained from a local supplier. During the first 7 days, the light regimen was continuous, which was reduced to 23 h of light afterward. The temperature was set initially at 32°C and gradually reduced by 3°C/7 days until 22°C was reached. Diets were formulated to meet nutrient requirements according to Japanese quail nutrient requirements per NRC (1994) recommendation for growing phase. 125 Japanese quails at 45 days of age were distributed in a completely randomised design, consisting of 5 treatments, 5 replicates and 5 (4 female and 1 male) birds per replicate. A period of 20 days was provided for the birds to adapt to the basal diets, cages and to thermoneutral environmental temperature (in heat-stressed groups temperature gradually raised by 4°C/7 days until 34°C was reached). The relative humidity was maintained between 55% and 60%. Birds were exposed to photoperiod including 16 L and 8 D. At 65 days of age quails were divided into four groups. A group of 25 quails was exposed to 22°C (thermoneutral group). Four groups (100 quails) were exposed to 34°C for 8 h/day according to Mujahid et al. (2006) as heat-stressed groups and allocated to 4 dietary treatments including heat-stressed and heat-stressed plus basal diet supplemented with arginine to provide arginine concentrations of 1.00, 1.15, 1.30 and 1.45 times the quail’s requirements per NRC (1994) recommendation (with a fixed concentration of digestible lysine (1.01%), resulting in arginine:lysine ratios of 1.247, 1.434, 1.621, and 1.808, respectively). The thermoneutral (TN) and heat-stressed groups were divided into separate rooms. Birds were housed in galvanised wire cages equipped with feeders and nipple drinkers. Each cage provided a space of 0.20 m²/5 birds.

The basal diet was formulated mainly based on corn, soybean meal and corn gluten meal (Table 1) and satisfied recommendations of NRC (1994) requirements. During the entire experimental period, the quails had free access to water and feed.

Performance trial of breeder quails

Egg production, feed intake, egg weight, feed conversion ratio and eggshell, yolk and albumen percentage were evaluated. Egg production was determined by daily egg collection, which was recorded on one spreadsheet per replicate. At 2, 4, 6 and 8 weeks of the assay, all eggs produced in a single day were collected for quality evaluation (average weight, albumen and yolk percentages, eggshell thickness and Haugh unit). Average albumen weight per replicate was determined as the difference between average egg weight and average yolk weight plus eggshell weight per replicate. In order to determine the eggshell weight, eggs were cracked and the shells were washed and dried at room temperature for 48 h prior to being weighed on an analytical balance (GE120, A&D Weighting, Made in South Korea). Shell thickness
was measured by a micrometer (Mitutoyo, Made in Japan) as an average of 3 points (top, medial and base). Haugh unit (HU) calculated according to Brant (1980) method. Briefly, an equal volume of buffer (0.01 M sodium phosphate, 0.1 M NaCl, pH 7.5) was added to the yolk and stirred. Solid polyethylene glycol 6000 (Sigma-Aldrich) was added to a concentration of 3.5%, stirred until dissolved, and the resultant protein precipitate was pelleted by centrifugation at 10,000 × g for 15 min. The supernatant was filtered through filter paper and decanted into another centrifuge tube for further purification of IgY. Next, 12% w/v solid polyethylene glycol (PEG) was added to the supernatant, stirred thoroughly and centrifuged at 10,000 × g for 15 min, in order to precipitate IgY. The pellet was redissolved to the original yolk volume in 0.01 M sodium phosphate buffer, 0.1 M NaCl, pH 7.5, and then PEG was added to 12% w/v for second precipitation. The supernatant was then decanted and the pellet was centrifuged twice more to remove any residual PEG trapped in the precipitate. This final IgY pellet was then dissolved in phosphate buffer (0.01 M, pH 7.5) to the original volume of yolk and stored at −20°C.

The total IgG levels in the maternal serum, IgY in the egg yolks and day-old chick’s serum IgG were determined using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions according to Laemmli (1970). The resultant from the IgY precipitation steps were dissolved in the sample buffer with 2% 2-mercaptoethanol and run on a 5% stacking gel and 10% separating gel. Samples and standards were loaded on the gel at a concentration of 20 μg. The gel was run at 20 mA for 1.5 h and stained with Coomassie Brilliant Blue. Densitometric analysis of the IgG and IgY was performed using RFLPscan software (Strategene, California, USA).

**Immune assay**

**Egg yolk IgY purification and immunoglobulin determination**

IgY was isolated from egg yolks using Polson et al. (1980) method. Briefly, an equal volume of buffer (0.01 M sodium phosphate, 0.1 M NaCl, pH 7.5) was added to the yolk and stirred. Solid polyethylene glycol 6000 (Sigma-Aldrich) was added to a concentration of 3.5%, stirred until dissolved, and the resultant protein precipitate was pelleted by centrifugation at 10,000 × g for 15 min. The supernatant was filtered through filter paper and decanted into another centrifuge tube for further purification of IgY. Next, 12% w/v solid polyethylene glycol (PEG) was added to the supernatant, stirred thoroughly and centrifuged at 10,000 × g for 15 min, in order to precipitate IgY. The pellet was redissolved to the original yolk volume in 0.01 M sodium phosphate buffer, 0.1 M NaCl, pH 7.5, and then PEG was added to 12% w/v for second precipitation. The supernatant was then decanted and the pellet was centrifuged twice more to remove any residual PEG trapped in the precipitate. This final IgY pellet was then dissolved in phosphate buffer (0.01 M, pH 7.5) to the original volume of yolk and stored at −20°C.

**Table 1. Ingredients and chemical composition of the basal diet fed to breeder quails.**

| Ingredient                                           | g/100 g (as fed basis) |
|------------------------------------------------------|------------------------|
| Corn                                                 | 55.27                  |
| Soybean meal                                         | 32.50                  |
| Gluton meal                                          | 1.61                   |
| Vegetable oil                                        | 2.99                   |
| Carbonate calcium                                    | 5.64                   |
| Dicalcium phosphate                                  | 1.14                   |
| Common salt                                          | 0.34                   |
| Mineral premix                                        | 0.25                   |
| Vitamin premix                                       | 0.25                   |
| Calculated analysis                                  |                        |
| ME, Kcal/Kg                                          | 2900.00                |
| Crude protein, %                                     | 20.00                  |
| Calcium, %                                           | 2.50                   |
| Available Phosphorus, %                              | 0.35                   |
| Sodium, %                                            | 0.15                   |
| Arginine, %                                          | 1.26                   |
| Lysine, %                                            | 1.01                   |
| Argininelysine ratios                                 | 1.24                   |
| Methionine + Cysteine, %                              | 0.70                   |

4Mixture supplied per kg of diet: retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; dl-tocopheryl acetate, 1.25 mg; mandelione sodium bisulphite, 2.5 mg; thiamine-hydrochloride, 1.5 mg; riboflavin, 3 mg; D-pantothenic acid, 5 mg; pyridoxine hydrochloride, 2.5 mg; vitamin B12, 0.0075 mg; folic acid, 0.25 mg; niacin, 12.5 mg.

5Mixture supplied per kg of diet: Mn (MnSO4·H2O), 50 mg; Fe (FeSO4·7H2O), 30 mg; Zn (ZnO), 30 mg; Cu (CuSO4·5H2O), 5 mg; I (KI), 0.025 mg; dl-tocopheryl acetate, 1.25 mg; menadione sodium bisulphite, 0.1 mg; choline chloride, 125 mg.

6ME: Metabolisable energy.

Approximately 250 eggs (50 eggs per treatment) were collected over 3 day period in week 8. All eggs were incubated to determine hatchability. Eggs were stored at 15°C before incubation. The setter was operated at a temperature of 37.70°C and relative humidity of 60% during the first 14 days of incubation and eggs were turned after every hour. On day 15 of incubation, eggs of each replicated tray (experimental eggs) were transferred to separate hatch baskets and placed in a hatch for the final 3 days. The temperature was decreased to 37.20°C, and the relative humidity was increased to 70%.

Unhatched eggs were analysed for embryo mortality causes, which were classified as early, middle and late dead. In order to determine offspring serum IgG, all 1-day old chicks were hatched from each replicate, bled by cardiac puncture, and blood was collected. The serum was then separated by centrifugation at 3000 × g for 15 min (serum from each replicate was pooled). Samples were stored at −20°C.

**Cutaneous basophil hypersensitivity**

The cellular immune response was assessed by a cutaneous basophil hypersensitivity test using phytohaemagglutinin (PHA). At day 30 and day 45 of the experiment, one quail from each replicate was selected, and the toe thickness of both left and right feet at the 3rd and 4th inter-digital spaces was measured by micrometer. Immediately following the measurements, 0.05 ml PHA (1 mg PHA/ml in PBS) and 0.05 ml of phosphate-buffered saline (PBS) were injected intradermally into the foot webbing of the 3rd and 4th digits of the right and left foot of each bird (left to act as the control), respectively. The web
swelling of both feet was measured 12, 24 and 36 h after injection. The response was determined by subtracting the skin thickness of the first measurement from the second measurement, and values of the left foot (control) were subtracted from those of the right foot (Corrier and Deloach 1990).

**Antibody titre to sheep red blood cells**

Humoral immune response was evaluated by haemagglutination (HA) antibody titre estimation. A suspension of sheep red blood cells (SRBC) (1% and 2.50% v/v) in PBS was prepared and stored under refrigeration at 4°C until use. At days 30 and 45 of the experiment, 0.20 mL of 2.50% SRBC suspension was injected intramuscularly per quail, and a total of 5 birds were injected per treatment in order to study the primary antibody response to SRBC. At days 37 and 52 respectively, for each SRBC injection, 1 ml of blood was collected from the wing vein. The blood was allowed to clot, and the serum was collected, and frozen (−20°C) until analysed for antibody titres to SRBC. The antibody titre was determined by the HA test and titres were expressed as log₂ (Siegel and Gross 1980).

**Assay of antioxidant indices and plasma lipids**

At 60 days of assay, one quail from each replicate were randomly selected and taken to the animal house slaughter facility. Liver samples were taken immediately after slaughter and homogenised in freezing isotonic physiological saline to form homogenates at the concentration of 0.10 g/mL. The liver samples were obtained to measure malondialdehyde (MDA) and antioxidant enzymes activity.

Blood samples were collected from the wing vein into EDTA-coated syringes and plasma was separated by centrifugation at 3000 × g for 15 min at room temperature and stored in aliquots at −20°C until use. The plasma samples were measured for MDA levels and for superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and total antioxidant capacity (TAC).

Malondialdehyde levels (Pegg 1986) and SOD (Yang et al. 2016), CAT (Adams et al. 1991), and GPx (Heinzen and Pollack 2003) were measured by using spectrophotometric methods (Hitachi U-2001 spectrophotometer, Tokyo, Japan) as described in the literature.

**Statistical analysis**

The obtained data were submitted to analysis of variance using the General Linear Model (GLM) procedure of the SAS statistical package (SAS Institute 2002). Significant difference among means of treatments was detected by Duncan’s multiple range test procedures. The differences were considered significant at (p < .05). The effect of arginine supplementation in heat stress conditions was assessed using orthogonal polynomial contrast for linear and quadratic effects. The following model was used to study the effect of test materials on parameters investigated as follows:

\[ y_{ij} = \mu + T_i + e_{ij} \]

\[ y_{ij} = \text{Observation for each dependent variable.} \]
\[ \mu = \text{Overall mean.} \]
\[ T_i = \text{Treatment effects.} \]
\[ e_{ij} = \text{Random residual effects.} \]

**Results**

**Productive performance**

The effect of HS on breeder quails was observed as a decrease in egg weight (EW), egg production (EP) and daily feed intake (DFI) in the HS group as compared to the thermoneutral (TN) group (Table 2). Arg supplementation increased EW, EP and DFI and improved FCR (p < .05) compared to the basal group exposed to HS. Supplementation of quails diet with extra Arg improved (p < .05) EP, EW, and DFI similar to those of the TN group. There were quadratic increases in EW with increasing addition of Arg (p < .05). The EP, DFI increased while the FCR decreased linearly and quadratically as graded increments of Arg were added to the diet (p < .05).

**Egg quality parameters**

There were no significant (p < .05) differences in the yolk and albumen percentages and eggshell thickness across all experimental groups (Table 2). The results showed that HU as an index of albumen quality, significantly (p < .05) decreased in the HS group fed basal diet as compared to the TN group. The results indicated that the HU score in groups that fed the experimental diets was significantly (p < .05) increased compared with the HS group. The HU score in the Arg supplementation groups was similar to those of the birds in the TN group. There were linear increases in the yolk and albumen percentages and HU, and
Table 2. Effect of arginine supplementation on productive performance, egg quality and reproductive performance in breeder quails.

| Parameter                  | Thermoneutral | Basal diet | 1.15 Arg¹ | 1.3 Arg | 1.45 Arg | SEM² | ANOVA Linear Quadratic | p-Value |
|----------------------------|---------------|------------|-----------|---------|----------|------|------------------------|---------|
| Productive performance     |               |            |           |         |          |      |                        |         |
| Egg weight, g              | 11.64         | 11.45      | 12.42     | 12.56   | 11.95    | 0.022| <.0001                 | .1073   | <.0001                 |
| Egg production, %          | 77.22         | 71.94      | 82.16     | 81.00   | 76.50    | 0.503| <.0001                 | .0160   | <.0001                 |
| Daily feed intake, g       | 25.80         | 23.37      | 25.21     | 25.82   | 23.86    | 0.107| <.0001                 | .0291   | <.0001                 |
| FCR                       | 3.61          | 3.70       | 3.24      | 3.33    | 3.45     | 0.041| <.0001                 | .0155   | <.0001                 |
| Egg quality                |               |            |           |         |          |      |                        |         |
| Yolk, %                    | 31.73         | 30.55      | 31.91     | 31.81   | 31.99    | 0.343| .0683                  | .0224   | .4606                  |
| Albumen, %                 | 56.98         | 58.32      | 58.43     | 58.52   | 58.23    | 0.452| .0814                  | .0248   | .3926                  |
| Eggshell thickness, mm     | 0.170         | 0.166      | 0.172     | 0.170   | 0.171    | 0.001| .4884                  | <.0001  | <.0001                 |
| Haugh unit                 | 85.32         | 81.52      | 85.44     | 85.23   | 85.42    | 0.206| <.0001                 | .0192   | .5762                  |
| Reproductive parameters    |               |            |           |         |          |      |                        |         |
| Fertility, %               | 90.22         | 80.74      | 89.65     | 90.91   | 87.33    | 1.005| .0199                  | .0860   | .144                   |
| Hatchability, %            | 75.59         | 65.47      | 77.14     | 75.50   | 74.69    | 1.137| .0211                  | .0004   | .0684                  |
| Embryonic mortality, %     |               |            |           |         |          |      |                        |         |
| Early dead                 | 2.09          | 7.14       | 6.94      | 4.08    | 4.89     | 0.658| .2850                  |         |                        |
| Middle dead                | 8.93          | 8.93       | 7.34      | 7.75    | 6.94     | 0.783| .9119                  | .1818   | .7445                  |
| Late dead                  | 12.50         | 18.40      | 11.40     | 12.65   | 11.00    | 0.943| .1381                  | .1602   | .4518                  |
| Total dead                 | 24.40         | 34.50      | 22.80     | 24.49   | 25.70    | 1.142| .0227                  | .0454   | .2525                  |

¹Arginine. ²SEM: Standard error of means. *Means within rows with different superscripts are significantly different (p < .05).

Linearly and quadratically increases in eggshell thickness with increasing of Arg supplementation (p < .05).

Fertility, hatchability and embryonic mortality

As shown in Table 2, fertility and hatchability decreased (p < .05) in quails under HS compared with quails under TN conditions. Quails exposed to HS and fed with Arg supplementation diets showed higher (p < .05) fertility and hatchability than those fed the basal diet and exposed to the same HS condition. Arg inclusion in the diet of heat-stressed quails resulted in similar (p < .05) fertility and hatchability to those under TN conditions. There were linear increases in the hatchability and quadratically increases in fertility with increasing concentration of Arg in diets (p < .05).

There was no significant (p < .05) difference in early, middle, and late embryonic mortality across all experimental groups. However, quails exposed to HS had higher (p < .05) overall embryonic mortality compared with quails in the TN group. All quails receiving the Arg in the diet had lower (p < .05) total embryonic mortality compared with the quails fed the basal diet and exposed to the same HS condition. Supplementation of quails diet with Arg resulted in similar (p < .05) total embryonic mortality with those of reared under TN condition.

Immune assay

Maternal serum immunoglobulin, IgY and IgG transfer to offspring

Results were cited in Table 3 show that the maternal serum IgG, egg yolk IgY, offspring serum IgG and IgG transfer rate to offspring in breeder quail fed diets supplemented with Arg under hot environmental stress. Results indicated that maternal serum IgG, egg yolk IgY, offspring serum IgG and IgG transfer rate to offsprings significantly (p < .05) decreased in the quails fed the basal diet and exposed to HS as compared to the TN group. The results indicated that maternal serum IgG, egg yolk IgY and offspring serum IgG in groups that fed the experimental diets (HS + Arg groups) increased significantly (p < .05) compared with the HS group. The maternal serum IgG, egg yolk IgY and offspring serum IgG in HS plus Arg concentration 1.15, 1.30, and 1.45 times the quail’s NRC (1994) recommendations groups were similar to those of birds in the TN group. There were linear increases in the maternal serum IgG, egg yolk IgY, offspring serum IgG and IgG transfer rate to offspring with increasing of Arg supplementation (p < .05).

Humoral and cell mediate immune

The data in Table 3 represents the humoral immune responses and cell-mediated immune responses of Japanese breeder quails. Birds reared under HS conditions showed a significant (p < .05) reduction in antibody titres against SRBC during primary and secondary responses as compared with TN treatment. The results indicated that dietary Arg significantly increased titres of antibodies for a secondary response when birds were exposed to HS conditions (p < .05), whereas antibody titres against SRBC were not affected by the Arg as compared with the TN treatment (p < .05).

The cell-mediated immune response to PHA was reduced (p < .05) by HS. Supplementation of Arg
Table 3. Effect of arginine supplementation on maternal and offspring IgG and egg yolk IgY, humoral (log2) and cell immune response in breeder quails.

| Parameter                        | Thermoneutral | Basal diet | 1.15 Arg | 1.30 Arg | 1.45 Arg | SEM | ANOVA | Linear | Quadratic |
|----------------------------------|---------------|------------|----------|----------|----------|-----|-------|--------|-----------|
| Maternal and offspring serum IgG and egg yolk IgY | MEAN±SE         |           |         |          |          |     |       |        |           |
| Maternal plasma IgG, mg/mL       | 11.80±0.35     | 11.02±0.39| 10.30±0.29| 10.20±0.30| 10.10±0.31| 0.30| 0.0022| 0.0004| 0.3954    |
| Egg Yolk IgG, mg/mL              | 5.76±0.25      | 5.40±0.23 | 5.16±0.21 | 5.00±0.20 | 4.80±0.19 | 0.28| 0.0001| 0.0001| 0.0001    |
| Offspring Serum IgG, mg/mL       | 3.80±0.25      | 3.40±0.23 | 3.16±0.21 | 3.00±0.20 | 2.80±0.19 | 0.20| 0.0001| 0.0001| 0.0001    |
| IgG transfer, %                  | 32.20±2.39     | 30.20±2.37| 28.20±2.35| 26.20±2.33| 24.20±2.31| 2.31| 0.0001| 0.0001| 0.0001    |
| SRBC                             |               |           |          |          |          |     |       |        |           |
| Primary injection                | 2.66±0.23      | 2.40±0.21 | 2.20±0.19 | 2.00±0.18 | 1.80±0.17 | 0.17| 0.0001| 0.0001| 0.0001    |
| Secondary injection              | 5.00±0.25      | 4.80±0.24 | 4.60±0.23 | 4.40±0.22 | 4.20±0.21 | 0.21| 0.0001| 0.0001| 0.0001    |
| PHA, mm                          |               |           |          |          |          |     |       |        |           |
| Primary PHA response             |               |           |          |          |          |     |       |        |           |
| 12 h                             | 0.17±0.01      | 0.11±0.01 | 0.18±0.02 | 0.20±0.03 | 0.28±0.06 | 0.06| 0.0001| 0.0001| 0.0001    |
| 24 h                             | 0.16±0.01      | 0.10±0.01 | 0.17±0.02 | 0.19±0.03 | 0.21±0.06 | 0.06| 0.0001| 0.0001| 0.0001    |
| 36 h                             | 0.15±0.01      | 0.09±0.01 | 0.17±0.02 | 0.18±0.03 | 0.18±0.06 | 0.06| 0.0001| 0.0001| 0.0001    |
| Secondary PHA response           |               |           |          |          |          |     |       |        |           |
| 12 h                             | 0.19±0.01      | 0.12±0.01 | 0.21±0.02 | 0.22±0.03 | 0.22±0.06 | 0.06| 0.0001| 0.0001| 0.0001    |
| 24 h                             | 0.18±0.01      | 0.11±0.01 | 0.18±0.02 | 0.20±0.03 | 0.21±0.06 | 0.06| 0.0001| 0.0001| 0.0001    |
| 36 h                             | 0.16±0.01      | 0.09±0.01 | 0.18±0.02 | 0.18±0.03 | 0.21±0.06 | 0.06| 0.0001| 0.0001| 0.0001    |

1Arginine. 2The percentage transfer of IgG from the dams’ plasma to the plasma of 1-day-old chicks was calculated by dividing the 1-day-old chicks’ plasma antibody levels by the dams’ plasma antibody levels and multiplying this value by 100. 3Phytohaemagglutinin. 4SEM: Standard error of means. a–cMeans within rows with different superscripts are significantly different (p < .05).

Table 4. Effect of arginine supplementation on antioxidant parameters and malondialdehyde in plasma and liver of breeder quails.

| Parameter                        | Thermoneutral | Basal diet | 1.15 Arg | 1.30 Arg | 1.45 Arg | SEM | ANOVA | Linear | Quadratic |
|----------------------------------|---------------|------------|----------|----------|----------|-----|-------|--------|-----------|
| **Plasma**                       |               |           |          |          |          |     |       |        |           |
| SOD, unit/mL                     | 165±5.0       | 155±6.0   | 194±6.0  | 188±6.0  | 168±6.0  | 3.24| 0.0001| 0.0001| 0.0242    |
| CAT, unit/mL                     | 6.51±0.3      | 4.70±0.3  | 7.48±0.3 | 7.50±0.3 | 6.50±0.3 | 0.20| 0.0001| 0.0001| 0.0001    |
| GPx, unit/mL                     | 188±6.0       | 175±6.0   | 230±6.0  | 220±6.0  | 195±6.0  | 4.54| 0.0001| 0.0001| 0.0041    |
| MDA, nmol/mL                     | 3.43±0.3      | 3.71±0.3  | 3.37±0.3 | 3.32±0.3 | 3.24±0.3 | 0.03| 0.0001| 0.0001| 0.0017    |
| TAC, nmol/mL                     | 5.90±0.3      | 4.11±0.3  | 5.37±0.3 | 6.32±0.3 | 6.74±0.3 | 0.19| 0.0001| 0.0001| 0.0001    |
| **Liver**                        |               |           |          |          |          |     |       |        |           |
| SOD, nmol/mg protein             | 122±4.0       | 114±4.0   | 156±4.0  | 144±4.0  | 121±4.0  | 2.81| 0.0001| 0.0001| 0.3931    |
| CAT, nmol/mg protein             | 22.3±2.0      | 16.9±2.0  | 24.1±2.0 | 23.7±2.0 | 22.1±2.0 | 0.54| 0.0001| 0.0001| 0.0001    |
| GPx, nmol/mg protein             | 21.7±2.0      | 19.2±2.0  | 26.6±2.0 | 24.9±2.0 | 21.4±2.0 | 0.60| 0.0001| 0.0001| 0.7258    |
| MDA/nmol/mg protein              | 0.98±0.3      | 1.47±0.3  | 1.34±0.3 | 1.40±0.3 | 1.45±0.3 | 0.03| 0.0001| 0.0001| 0.0826    |

1Arginine. 2Superoxide dismutase. 3Catalase. 4Glutathione peroxidase. 5Malondialdehyde. 6Total antioxidant capacity. 7SEM: Standard error of means. a–cMeans within rows with different superscripts are significantly different (p < .05).

Increased (p < .05) stimulation indices 12, 24 and 36 h after the injection of PHA compared with the basal group exposed to HS and resulted in a similar response to PHA compared with quails under TN condition. There was a linear improvement in the humoral immune responses and cell-mediated immune responses with increasing concentration of Arg in diets (p < .05).

**Antioxidant enzyme activities and lipid peroxidation**

Table 4 shows the effects of dietary treatment on plasma and liver antioxidant indices. The plasma and liver activities of SOD, CAT, and GPx were lower (p < .05) in the HS group compared with the TN group, whereas the reverse was true for malondialdehyde concentrations. At the dietary supplementation with Arg, increased (p < .05) the plasma and liver activities of SOD, CAT and GPx, and reduced (p < .05) the concentrations of MDA. In addition, administration of 1.15 times Arg requirements NRC (1994) recommended to the diet resulted in higher (p < .05) plasma and liver activities of SOD, CAT, and GPx and lower levels of MDA and TAC of plasma compared with the TN group. Breeder quails receiving the Arg diet and those reared under TN condition exhibited higher (p < .05) plasma TAC compared with the HS and TN groups. In plasma, treatment with the increasing levels of Arg caused a linear and quadratic decrease in MDA contents and increased SOD, CAT and GPx activities and TAC in a linear and quadratic fashion (p < .05).

Also, in the liver were a linear decrease in MDA contents and linearly increases in SOD, CAT and GPx activities with increasing of Arg supplementation level (p < .05).
Discussion

In the current study, high environmental temperature reduced the DFI, EP, EW, and egg quality which is in agreement with previous findings (Table 2). The reduced EP and EW could be attributed in part to the decrease in feed intake and to an impairment in the utilisation of nutrients. Heat stress could also negatively impact nutrient utilisation in birds by changes in the intestinal morphology, where different intestinal segments (duodenum, jejunum, and ileum) exhibit lesions that vary in their degree, as well as differences in their relative weight, villus height, villus surface area, crypt depth (He et al. 2018) and by reducing the activity of digestive enzymes (Wu et al. 2004). Heat stress also causes oxidative stress, as a primary factor in decreased production performance in birds (Mujahid et al. 2006).

In our study, we investigated the Arg to protect breeder quails against HS. Diet supplementation with 1.15, 1.30 and 1.45 times of Arg requirement restored the impairment in DFI, EP, EW and egg quality in quails submitted to HS. Our finding regarding the anti-stress property of Arg is in agreement with those reported by other researchers in laying hens under HS (Bozakova et al. 2015). The reduced EP and egg quality in heat-exposed quails might be due to the reduction in feed intake and impairment in the utilisation of nutrients. The positive effects of arginine supplementation were attributed to the inhibiting role of its metabolite nitric oxide (NO) on ACTH and corticosterone secretion, the suppression of adrenal glucocorticoid synthesis and arginine antioxidant activity (Gupta et al. 2005). Improved of EP and EW in heat-stressed quails receiving Arg could be attributed to the increase in feed intake (Table 2) and improvements of intestinal function, such as intestinal morphology, energy status, antioxidative capacity, and intestinal digestion (Bozakova et al. 2015).

Ornithine (Arginine-related compound) led to a significantly higher crypt height in the jejunum and ileum and a higher total villous height in the ileum. Ornithine is converted into putrescine by ornithine decarboxylase. Putrescine is converted into spermidine by the action of an aminopropyl transferase. The functions of polyamines in cells are poorly understood, although the use of ornithine decarboxylase inhibitors such as difluoromethyl ornithine has established that polyamines are essential for cell growth (Pegg 1986) and protein synthesis (Grillo 1985). In this way, it has been clearly shown that the action of ornithine on fibroblast growth in culture (Uni and Ferket 2004) and on protein synthesis by the liver (Oratz et al. 1983) are both dependent upon polyamine synthesis. With regard to the intestine, there are consistent data supporting an important role of polyamines in the control of hypo and hyperplasia of this tissue. Ornithine decarboxylase in the mucosa of the small intestine has high basal activity compared with most tissues (Pegg 1986). Ornithine decarboxylase is associated with mature cells of the villus tip as well as proliferating crypt cells, suggesting that polyamines participate in both intestinal cell differentiation and proliferation (Johnson et al. 1989).

In addition, our results showed that mean EP and EW was increased in quails fed with Arg supplementation diet compared to those fed basal diet under HS. Nitric oxide has been postulated to regulate follicular development, ovulatory mechanisms and egg production (Manwar et al. 2006). There is an association between the size of ovarian follicles and NO metabolites, nitrite (NO₂⁻) and nitrate (NO₃⁻), levels in serum and hypothalamus of laying quails (Manwar et al. 2006).

According to another report, HU is a measure most commonly used to express albumen quality. In our study, quails exposed to HS with basal diet had a lower HU score compared with quails reared under TN conditions. Dietary supplementation with Arg significantly improved the HU score of breeder quails exposed to HS to a level similar to that of quails reared under TN conditions. The internal quality of eggs can be influenced by numerous factors, including biological characteristics, nutrition and the environment (e.g. high temperature). Heat stress which may have been too low to achieve adequate albumen density could also explain the moderate HU values observed. Thus, it can be deduced that Arg supplementation in breeder quails diets under HS condition was sufficient to improve the quality of albumen through HU score to a level similar to that of quails reared under TN condition.

In our study, quails were exposed to HS and fed with Arg supplementation diets showed higher fertility and hatchability than those fed the basal diet and exposed to the same HS condition. Our finding is in agreement with those reported by Silva et al. (2012) and Ahangar et al. (2017) who observed that Arg supplementation had a favourable effect on egg production and hatchability in breeders. In this regard, Arg could be potentially used to enhance the fertility of broiler breeder hens due to the sperm penetration in the perevitelline membrane (Sharideh et al. 2016). Hellstrom et al. (1994) showed that NO substance had...
a regulatory effect on sperm motility and viability and consequently sperm fertilising potential.

Results of this study showed that the embryonic mortality of breeder quails was significantly ($p < 0.05$) decreased by dietary supplementation with Arg in the HS group. In this context, the reduction in mortality in Arg groups quail may also be attributed to improved immunity as discussed below.

In addition to impairing performance, HS also affects the immune response in birds. In the current study, Arg improved the maternal serum IgG, egg yolk IgY and offspring serum IgG in comparison with the study, Arg improved the maternal serum IgG, egg yolk IgY and offspring serum IgG in comparison with the basal group exposed to thermal stress. Xu et al. (2018) reported that dietary arginine supplementation in broiler chickens increased serum concentrations of IgA, IFN-γ, thymus weight, lymphocyte proliferation, antibody titres to Newcastle disease, and serum IgM concentration. Immunomodulatory actions of arginine are mediated by polyamines which are produced as one of the products of arginine; thus, polyamines increase lymphocyte mitogenesis and arginine-dependent macrophage-mediated tumour cell cytotoxicity (Evo et al. 1998). In addition, our results are in agreement with the findings of other researchers (Yi et al. 2016), which reports that the yolk colour and IgY content of the eggs from hens supplemented with 17 mg/kg L-arginine increased compared with that in the control group, which may be explained by the promotion of lutein and IgY deposition by dietary arginine. Therefore, it was an effective method to produce a functional egg-containing large quantity of IgY, which could be used to prevent diseases and embryonic mortality. Our finding shows a higher level of Arg to heat-stressed quails resulted in a higher level of immunoglobulin in breeder quails and offspring’s serum, as well as egg yolk than quails reared under TN condition. The highest antibody transmission rate from breeder quails to offspring also belonged to quails received the 1.15 times of Arg requirement. This observation suggests that the amount of maternal antibodies present in the chicks is ultimately decided by the levels in the dam. In agreement with our results, other researchers reported that the amount of IgG transported is independent of egg size and known to be proportional to the maternal serum IgG concentration (Loeken and Roth 1983).

In the present study, the humoral and cell-mediated immune responses were suppressed under the HS condition. It is well known that environmentally stressed poultry generally have a depressed humoral immune response. It has been reported that Arg plays an important role as a potent immunological modulator through the production of nitric oxide (Collier and Vallance 1989), which is involved in modulating T-cell mitogenic response.

Hypersensitivity skin test has been widely used as in vivo screening test due to its practicability and simplicity (Blaese et al. 1973). In agreement with our results (Abdukalykova and Ruiz-Feria 2006), showed that the supplementation of Arg significantly affected the CBH response in broilers. Dietary Arg improves thymus weight and function and enhances lymphocyte mitogenesis (Efron and Barbul 1998).

Heat stress could also induce oxidative stress resulting in an imbalance in antioxidant status in birds (Lin et al. 2006). Under HS conditions, as the bird’s body attempts to maintain its thermal homeostasis, increased levels of reactive oxygen species (ROS) occur. Consequently, tissues and cells possess defense mechanisms to detoxify ROS by radical scavengers, such as SOD, CAT and GPx (Wu et al. 2004). The results of our study are in agreement with the data available in the literature. In the present study, the activities of SOD, CAT, and GPx were lower and MDA concentration in serum was higher in quails exposed to HS. Dietary Arg supplementation increased the serum and liver activities of antioxidant enzymes such as SOD, CAT and GPx, and reduced the concentrations of MDA, an indirect parameter of lipid peroxidation and overproduction of ROS, in heat-stressed quails. The antioxidant effect of Arg was due to the increased level of glutathione (GSH) and glutathione/glutathione disulphide (GSSG) ratio and decreased lipid peroxidation in exercise-induced oxidative stress (Lin et al. 2006). Arginine is involved in the regulation of NADPH which is used in the production of GSH from GSSG (Flynn et al. 2002). It was conducted that Arg has strong radical scavenging activity against oxygen radicals (Lass et al. 2002). Nitric oxide is a known potential scavenger of superoxide radical leading to the inactivation of superoxide radical (Yi et al. 2016). It was suggested that the superoxide scavenging effect is due to the arginine-mediated increase in NO production (Yi et al. 2016).

Conclusions

In conclusion, the results of the present study demonstrated that breeder quails subjected to the HS showed deteriorated productive performance, reproduction, egg quality, antioxidant statuses and immune response. Dietary supplementation with either 1.15, 1.30 and 1.45 times of Arg requirement (as NRC recommendation) in breeder quails reared under HS
condition can alleviate the adverse effects of HS and improve the performance, reproduction, antioxidant statuses and immunity as well as maternal antibody transmission. In addition, administration of Arg to the diet restored the most parameters near to the TN group. Even, in some cases, the supplementation of diet with 1.15 times of Arg requirement resulted in better responses than quails under TN condition (Subsequently the best arginine:lysine ratios = 1.434). Therefore, it was concluded that Arg in a higher level of NRC (1994) recommendation can be used as a feed additive for the alleviation of the adverse effects caused by HS in breeder quails.

**Ethical approval**

All experimental protocols adhered to the guidelines of and were approved by the Animal Ethics Committee of the University of Kurdistan, Sanandaj, Iran.

**Disclosure statement**

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

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**Data availability statement**

The original data of the paper are available upon request from the corresponding author.

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