Comparison of growth performance and immune responses of broiler chicks reared under heat stress, cold stress and thermoneutral conditions

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Abstract

This study was conducted to compare the effects of thermal stress on growth performance and some immunity variables of broiler chickens. Birds were randomly assigned to one of three thermal treatments as follows: cold stress (CS, 12±1 °C), heat stress (HS, 33±3 °C) and thermoneutral (TN, 24±2 °C). Body weight gain (BWG), feed intake (FI), water intake (WI) and feed conversion ratio (FCR) were recorded. In order to evaluate the primary and secondary humoral immune responses, two birds per replicate were intravenously administrated with a suspension of 7% sheep red blood cell (SRBC) at 28 and 35 days. The heat-stressed broiler chickens had lower FI (-14.90%), BWG (-25.71%) and higher FCR (+13.06%) in comparison to broiler chickens reared under TN condition (p<0.001) from 1 to 42 days of age. The cold-stressed broiler chickens showed lower FI (-22.05%), BWG (-38.32%) and higher FCR (+22.47%) in comparison to birds reared under TN conditions (p<0.001). Stressed birds (CS and HS) showed decreased antibody titer against SRBC, lymphocyte count and the relative weights of lymphoid organs and increased heterophil count, heterophil to lymphocyte ratio and the serum concentration of corticosterone, in comparison to birds in TN group (p<0.001). In conclusion, HS and CS conditions have similar negative effects on performance and immunity of broiler chickens.

Additional keywords: antibody titer; broiler chicken; immunity; thermal stress.

Abbreviations used: BWG (body weight gain); CS (cold stress); FCR (feed conversion ratio); FI (feed intake); HS (heat stress); ME (2-mercaptoethanol); ROS (reactive oxygen species); SRBC (sheep red blood cell); TN (thermoneutral); WI (water intake).

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Introduction

Birds are usually faced with environmental stresses while bred. Stress is defined as a biological reaction of animals to environmental stimuli, being considered a major challenge in the poultry industry, because of its unfavorable effects on growth performance. Both intrinsic and extrinsic negative stressors change body homeostasis in animals. For instance, the Royal Society for the Prevention of Cruelty to Animals (RSPCA), in its welfare standards for meat chicken, has recommended monitoring animals for having access to a thermal comfortable environment at all times, preventing both heat (HS) and cold stress (CS). Moreover, the World Organization for Animal Health (OIE) standards recommends avoiding severe heat, humidity and cold (Stevenson et al., 2014). In order to prevent these environmental stresses, the OIE has also advised to employ higher air speed, evaporative cooling and reducing stocking density (Stevenson et al., 2014).

Zhang et al. (2011) have reported that CS (12 °C in this study) negatively influences the intestinal system in broiler chicken. CS have negative effects on digestibility (Onderci et al., 2003), health and comfort
in animals (Tsutsayeva & Sevryukova, 2001). CS also impairs egg production and feed efficiency in laying hens (Sahin et al., 2002). The CS not only decreases growth performance, but also increases energy requirements in broiler chicken (Blahova et al., 2007).

Similarly, HS (30 °C in this study) negatively affects the performance of birds (Yardibi & Turkay, 2008; Habibian et al., 2014; Tawfeek et al., 2014). Sohail et al. (2012) reported that broiler chickens submitted to chronic HS had lower feed intake (FI; -16.4%), body weight gain (BWG; -32.6%), and higher feed conversion ratio (FCR; +25.6%) in 42 days of age. HS has negative effects on decreasing growth performance, including decreased protein retention in muscles and increased heat production in broiler chickens (Zhang et al., 2012).

In addition to growth performance, immune responses may be negatively influenced by environmental stresses (Bartlett & Smith, 2003; Niu et al., 2009b).

The heterophil-to-lymphocyte ratio has been used as criteria for evaluating the stress, and Campo et al. (2008) showed higher heterophil-to-lymphocyte ratio and lower welfare in broiler chicken submitted to low ambient temperature. Moreover, exposure to stressors provokes neuroendocrine responses in the body and increases the levels of corticosterone (Iyasere et al., 2017). Corticosterone, an end product of the hypothalamic-pituitary-adrenal axis, has been reported to have regulating effects on metabolism, feed consumption, and immune system (Zulkifli & Sti Nor Azah, 2004). Corticosterone also causes a regression in lymphoid tissues in broiler chickens (Yang et al., 2015). Therefore, stress can have negative effects on performance and immunity by increasing the levels of corticosterone hormone.

Thus, this study aimed to compare the effects of CS, HS and thermoneutral (TN) treatments on growth performance and on some immune variables in broiler chickens.

Material and methods

Ethical standards

All the protocols used in this study were approved by the Institutional Animal Care and Use Committee of Tabriz University (Tabriz-Iran). We tried to minimize the pain or discomfort of the birds at all times.

Animals and temperature

The present study was conducted from December to February. A total of 180 broiler chicken (1 day old) were randomly allotted to three treatment groups (3 chambers), consisting of 6 replicates with 10 broiler chickens in each replicate. The recommended rearing temperature (33 °C) was applied during the first 3 weeks and they were progressively changed on day 22 of age. The broiler chickens were reared under HS, CS and TN conditions from days 22 to 42 of age. The birds were submitted to temperatures of 33±3 °C as HS (Moeini et al., 2012), 12±1 °C as CS (Zhao et al., 2014) and 23.9±2 °C as TN. Humidity was kept by 54.4±5.5% in all the chambers and chambers were equipped with mechanical ventilation and heating systems. Birds had ad libitum access to mash feed and fresh water. Diets were prepared as recommended by NRC (1994) for all the birds (Table 1). The AOAC standards were applied to evaluate the crude protein (AOAC, 2004). The BWG, FI and FCR were periodically measured. Water intake (WI) and mortality were recorded daily. Mortality was taking into account to calculate the growth performance. A 23/1 L/D photoperiod, with an average light intensity of 20 lx, was maintained for the entire experiment. Wood shaving was used on the floor of pens.

Immune system variables

On day 28, 1 mL of 7% sheep red blood cells (SRBC) suspension was administrated through the right wing vein to 2 chickens per replicate, and blood samples were collected on day 35. The samples were centrifuged at 1,500×g for 10 min and stored at -20 °C until evaluating the primary antibody response to SRBC. At the same day (day 35), 1 mL of 7% SRBC suspension was administrated to the same birds and blood samples were collected, centrifuged and stored as described for the first step. The samples were evaluated for antibody responses including immunoglobulin (Ig) G and M by the 2-mercaptoethanol (ME) procedure as described by Habibian et al. (2014). Thus, primary (IgG1 and IgM1) and secondary (IgG2 and IgM2) humoral immune responses against SRBC were measured. Antibody titers measured against SRBC were reported as the log2.

On day 32, blood samples were collected from other 2 birds per replicate in order to evaluate the heterophil and lymphocyte counts and their ratio, as described by Habibian et al. (2014). On day 42, other two birds per replicate were randomly selected, weighed and killed. Immune organs (bursa, spleen, and thymus) were weighed and relative weights were expressed as a percentage of body weight.

Corticosterone concentration

On days 21 and 42, two birds per replicate were selected and the blood samples (3 mL/bird) were collected and centrifuged at 2,500×g for 15 min. The
Growth performance and immune responses of broilers reared under different thermal conditions

Table 1. Composition of the broiler chicken diets (g/kg).

| Diet composition (g/kg)         | Starter (days 1-21) | Grower (days 22-42) |
|---------------------------------|---------------------|---------------------|
| Corn (8.5% crude protein)       | 647.1               | 699.5               |
| Soybean meal (48% crude protein)| 312.0               | 251.9               |
| Soybean oil                     | 0.00                | 5.00                |
| Calcium carbonate               | 13.6                | 13.0                |
| Dicalcium phosphate             | 16.0                | 15.7                |
| Salt                            | 4.6                 | 4.0                 |
| Mineral & vitamin premixa       | 5.0                 | 5.0                 |
| HCl-Lysine                      | 0.0                 | 4.4                 |
| DL-Methionine                   | 1.7                 | 1.5                 |
| Calculated chemical composition |                     |                     |
| Metabolizable energy (MJ/kg)    | 12.13               | 12.89               |
| Crude protein (g/kg)            | 210.5               | 190.0               |
| Calcium (g/kg)                  | 10.1                | 9.6                 |
| Available phosphorus (g/kg)     | 5.0                 | 4.8                 |
| Lysine (g/kg)                   | 11.6                | 13.3                |
| Methionine+Cysteine (g/kg)      | 8.5                 | 7.6                 |
| Na (g/kg)                       | 2.0                 | 1.5                 |
| K (g/kg)                        | 8.5                 | 7.8                 |
| Cl (g/kg)                       | 2.5                 | 2.3                 |
| Na+K−Cl (meq/kg)                | 221                 | 203                 |

*Mineral–vitamin premix provided the following per kilogram of diet: vitamin A, 9000 IU; vitamin D3, 2100 IU; vitamin E, 30 mg; nicotinic acid, 30 mg; vitamin B12, 0.12 mg; calcium pantothenate, 10 mg; vitamin K3, 5 mg; thiamin, 1.1 mg; riboflavin, 4.5 mg; vitamin B6, 2.0 mg; folic acid, 0.5 mg; biotin, 0.5 mg; Fe, 50 mg; Cu, 10 mg; Mn, 70 mg; Zn, 50 mg; Co, 0.2 mg; I, 1.0 mg; Se, 0.3 mg; butylated hydroxytoluene (BHT), 150 mg; monensin, 100 mg.

Results

Growth performance

Effects of the different treatments on growth performance of broiler chickens are presented in Table 2. Groups did not significantly differ for growth performance up to 21 days of age (p>0.05). Subsequently, the birds in the HS group showed a lower FI (-25.15%), BW (-40.25%), and higher FCR (+21.29%) and WI (+13.35%) in comparison to the birds reared under TN condition (p<0.001) during the growing period (22-42 days). Considering the whole rearing period (1 to 42 days of age), broiler chickens submitted to HS exhibited lower FI (-18.30%), BW (-26.92%), higher FCR (+11.05%) and WI (+11.17%) in comparison to the birds reared under TN condition (p<0.001).

Similar results were seen for the CS group. The broilers exposed to thermal stress had lower FI (-22.05%), BW (-38.32%) and higher FCR (+22.47%) in comparison to those reared under TN from 22 to 42 days of age (p<0.001). Considering the whole rearing period (1 to 42 days of age), the birds in CS group showed lower FI (-14.90%), BW (-25.71%) and higher FCR (+13.06%) in comparison to the broiler chickens reared in TN (p<0.001). CS and TN groups did not significantly differ for WI.

Immune system

Table 3 shows the results for the immune system variables. CS and HS showed similar effects on immune variables. Broiler chickens exposed to thermal stress had lower IgG1 (-38%), IgM1 (-25%), IgG2 (-25%), IgM2 (-40%), lymphocyte counts (-25%), and relative weights for spleen (-38%), bursa (-25%) and thymus (-29%) in comparison to TN birds (p<0.001). Birds exposed to stress yielded higher heterophil count (+28%) and heterophil-to-lymphocyte ratio (+45%) in comparison to birds in the TN group (p<0.001).

Corticosterone concentration

The serum concentration of corticosterone on day 21 did not significantly differ between groups (Figure 1). Subsequently, the serum concentration of corticosterone increased in birds submitted to stress in comparison to those kept under TN conditions (p<0.001).

Discussion

Our findings showed that the CS and HS conditions had similar negative effects on growth performance...
### Table 2. Effects of thermoneutral, heat stress and cold stress on feed intake, body weight, feed conversion ratio and water intake of broiler chickens.

| Variables                  | Treatments | Thermoneutral | Heat stress | Cold stress | SEM | p  |
|----------------------------|------------|---------------|-------------|-------------|-----|----|
| Feed intake (g)            |            |               |             |             |     |    |
| 1-21 days                  |            | 1420.10±87.87 | 1426.30±82.60 | 1485.20±0.74 | 11.73 | NS |
| 22-42 days                 |            | 1426.30±82.60 | 1485.20±0.74 | 1420.10±87.87 | 11.73 | NS |
| 1-42 days                  |            | 1485.20±0.74  | 1420.10±87.87 | 1426.30±82.60 | 11.73 | NS |
| Body weight (g)            |            |               |             |             |     |    |
| 1-21 days                  |            | 867.10±27.18  | 880.20±24.46 | 879.40±23.08 | 3.35 | NS |
| 22-42 days                 |            | 1858.30±114.94| 1110.30±159.07 | 1146.30±124.27 | 51.99 | ***|
| 1-42 days                  |            | 2726.30±102.62| 1992.30±163.96 | 2025.20±125.47 | 52.02 | ***|
| Feed conversion ratio (g/g)|            |               |             |             |     |    |
| 1-21 day                   |            | 1.63±0.09     | 1.62±0.08   | 1.68±0.09   | 0.01 | NS |
| 22-42 day                  |            | 2.07±0.06     | 2.63±0.28   | 2.67±0.20   | 0.04 | ***|
| 1-42 day                   |            | 1.93±0.03     | 2.17±0.09   | 2.22±0.10   | 0.02 | ***|
| Water intake (mL)          |            |               |             |             |     |    |
| 1-21 days                  |            | 1472.20±49.25 | 1500.20±130 | 1439.20±183.31 | 18.12 | NS |
| 22-42 days                 |            | 5555.30±160.19| 6411.20±255 | 5677.20±192.42 | 58.57 | ***|
| 1-42 days                  |            | 7027.20±161.56| 7911.20±278 | 7194.40±298.26 | 61.43 | ***|

Superscripts (a-b) show significant differences in each row \((p<0.05)\). SEM: standard error of means. NS: non-significant \((p>0.05)\); *** \(p<0.001\).

### Table 3. Effects of thermoneutral, heat stress and cold stress on the anti-SRBC antibody response \((\log_2)\), differential counts of heterophils, lymphocytes, the heterophil-to-lymphocyte ratio and the relative weight of lymphoid organs \((\text{percentage of BW})\) of broiler chickens.

| Variables                  | Treatments | Thermoneutral | Heat stress | Cold stress | SEM | p  |
|----------------------------|------------|---------------|-------------|-------------|-----|----|
| Antibody titer             |            |               |             |             |     |    |
| IgG1                       |            | 2.07±0.20\(a\) | 1.26±0.10\(b\) | 1.27±0.10\(b\) | 0.05 | ***|
| IgM1                       |            | 2.98±0.28\(a\) | 2.20±0.23\(b\) | 2.21±0.22\(b\) | 0.05 | ***|
| IgG2                       |            | 3.54±0.21\(a\) | 2.65±0.18\(b\) | 2.64±0.19\(b\) | 0.06 | ***|
| IgM2                       |            | 2.4±0.16\(a\) | 1.41±0.16\(b\) | 1.44±0.17\(b\) | 0.06 | ***|
| White blood cells          |            |               |             |             |     |    |
| Heterophils (H)            |            | 19.44±1.71\(b\) | 28.66±3.02\(a\) | 26.77±3.57\(a\) | 0.69 | ***|
| Lymphocytes (L)            |            | 73.33±2.11\(a\) | 56.88±2.94\(b\) | 55.33±3.18\(b\) | 1.16 | ***|
| H/L                        |            | 0.26±0.02\(a\) | 0.50±0.05\(b\) | 0.48±0.07\(b\) | 0.01 | ***|
| Lymphoid organs            |            |               |             |             |     |    |
| Spleen                     |            | 0.18±0.01\(a\) | 0.11±0.00\(b\) | 0.12±0.01\(b\) | 0.004 | ***|
| Bursa                      |            | 0.11±0.01\(a\) | 0.08±0.00\(b\) | 0.08±0.00\(b\) | 0.002 | ***|
| Thymus                     |            | 0.24±0.02\(a\) | 0.16±0.01\(b\) | 0.17±0.01\(b\) | 0.005 | ***|

Superscripts (a-b) show significant differences in each row \((p<0.05)\). SEM: standard error of means. *** \(p<0.001\).
Growth performance and immune responses of broilers reared under different thermal conditions

Digestibility (Zhang et al., 2012) while increasing corticosterone blood levels (Sahin et al., 2002). The reason for these effects is that stress alters the neuroendocrine system in poultry, by the activation of the hypothalamic-pituitary-adrenal axis, resulting in increased levels of corticosterone (Quinteiro-Filho et al., 2012). This is likely due to the adverse effects of corticosterone on the digestive system, nutrient utilization and digestibility, which subsequently have negative effects on performance. Indeed, corticosterone decreases food intake, which in turn decreases villus height in the intestine (Hu & Guo, 2008), decreases nutrient utilization, apparent digestibility of protein, and integrity of the intestinal epithelium (Hu & Guo, 2008). Moreover, corticosterone changes the protein metabolism and increases catabolism of structural proteins (Post et al., 2003; Lin et al., 2004). There are further proof of the link between heat stress, corticosterone and intestinal integrity. For instance, HS-exposed birds showed a reduced villus-height to crypt-depth ratio (Bozkurt et al., 2012; Deng et al., 2012). We found that the serum concentration of corticosterone was not different between groups on day 21, but it was increased in stressed groups on day 42. Therefore, the CS and HS effects on performance are likely mediated by an increased in the corticosterone levels.

Thermal stress decreased antibody titer against SRBC in our experiment, agreeing with previous reports (Bartlett & Smith 2003; Niu et al., 2009a,b; Mishra et al., 2011; Zhang et al., 2011; Collins et al., 2012; Fu et al., 2013; Habibian et al., 2014). HS suppresses the immune response by stimulating the hypothalamic production of corticotrophin-releasing factors (CRF) and increasing the levels of corticosterone (Quinteiro-Filho et al., 2012, 2017). Moreover, the increase of corticosterone induced by thermal stress subsequently decreases the levels of IgA and interferon gamma, mRNA expression of pro- and anti-inflammatory cytokines, as well as mRNA expression of avian β-defensin-4, avian β-defensin-6 and Toll-like receptor-2 (Quinteiro-Filho et al., 2017). Such physiological changes suppress the immune system and increase the penetration the bacteria through the intestinal lining. In addition, thermal stress exerts negative effects on the redox balance, exacerbating the production of reactive oxygen species (ROS) (Feng et al., 2008) and contributing to the permeability of the gut lining to bacteria. Quinteiro-Filho et al. (2012) indicated that the HS-mediated elevated corticosterone levels increased inflammation and translocation of Salmonella enterica in broiler chickens. Therefore, corticosterone is the main culprit in the observed effects of thermal stress in our study and others', by suppressing the immune system and decreasing food intake, inflammatory cytokines and altering the function of the intestine.

In agreement with our findings, other researchers have reported an increase in heterophil to lymphocyte ratio in stressed animals (Campos et al., 2008; Prieto & Campo, 2010; Felver-Gant et al., 2012). For instance, stressed laying hens presented lower intraepithelial lymphocytes and IgA-secreting cells in the intestinal tract (Deng et al., 2012). Glucocorticoid hormones reduce lymphocyte counts, because of the higher adherence to endothelial cells and circulating lymphocytes in response to these hormones (Dhabhar, 2002). Shimi et al. (2008) reported that chickens treated with corticosterone yielded a higher heterophil-to-lymphocyte ratio and also presented morphological alterations in heterophil size, shape, and granulation, and lymphocyte cytoplasmic properties. Moreover, similarly to our study, other studies have shown that thermal stress decreases the relative weight of lymphoid organs (Niu et al., 2009a; Quinteiro-Filho et al., 2012). The reduced relative weight of the lymphoid organs can be attributed to the corticosterone content. Yang et al. (2015) obtained a lower weight for the lymphoid organs of chickens treated with corticosterone. Glucocorticoids, i.e. corticosterone, prevent glucose transport in a variety of peripheral tissues (Yang et al., 2015), leading to a decrease in the organs weight. Moreover, glucocorticoids increase apoptosis in spleen in rats (Collier et al., 1998), which might contribute to these effects. Our data for corticosterone levels and associated effects support these previous observations.

In conclusion, CS and HS had similar adverse effects on the performance and immune system of broiler chickens, and these effects are likely to be mediated by an increase in the serum concentration of corticosterone. Therefore, the stress associated to the adverse thermal environment in the early development of broiler chickens exerts a very negative effect on the animals.
through well-known physiological mechanisms. Our findings might be useful for improving both the welfare and productivity of broiler chickens.

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Growth performance and immune responses of broilers reared under different thermal conditions

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