



# 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), (b) heat stress (HS, 33±3 °C) and (c) 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).

**Authors' contributions:** Data acquisition, analyzed the results, drafted and edited the manuscript and responsible for the entire project: AO and MA. Study design and revised the manuscript: AM and TS. Contributed to data analysis, revised the manuscript and reviewed the pertinent literature: FMP. All authors read and approved the final manuscript.

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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 log<sub>2</sub>.

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

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

	Starter (days 1-21)	Grower (days 22-42)
Diet composition (g/kg)		
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 premix <sup>a</sup>	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

<sup>a</sup>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 B 12, 0.12 mg; calcium pantothenate, 10 mg; vitamin K3, 5 mg; thiamin, 1.1 mg; riboflavin, 4.5 mg; vitamin 6, 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.

serum concentration of corticosterone was measured by a corticosterone enzyme-linked immunosorbent assay kit (Enzo Life Science Farmingdale Company, USA) as reported by Tachibana *et al.* (2007).

### Statistical analysis

The data were analyzed using the ANOVA procedure of SAS software (2003). Means were subsequently compared using Duncan's least significance multiple-range test. Results are expressed as means  $\pm$  standard error (S.E.). Differences were considered as significant if  $p < 0.05$ .

## 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 CS showed 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			SEM	p
	Thermoneutral	Heat stress	Cold stress		
Feed intake (g)					
1-21 days	1420.10±87.87	1426.30±82.60	1485.20±0.74	11.73	NS
22-42 days	3868.30±260.60 <sup>a</sup>	2895.20±321.94 <sup>b</sup>	3015.40±283.25 <sup>b</sup>	71.66	***
1-42 days	5288.30±247.19 <sup>a</sup>	4320.30±299.94 <sup>b</sup>	4500.30±279.82 <sup>b</sup>	70.58	***
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 <sup>a</sup>	1110.30±159.07 <sup>b</sup>	1146.30±124.27 <sup>b</sup>	51.99	***
1-42 days	2726.30±102.62 <sup>a</sup>	1992.30±163.96 <sup>b</sup>	2025.20±125.47 <sup>b</sup>	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 <sup>b</sup>	2.63±0.28 <sup>a</sup>	2.67±0.20 <sup>a</sup>	0.04	***
1-42 day	1.93±0.03 <sup>b</sup>	2.17±0.09 <sup>a</sup>	2.22±0.10 <sup>a</sup>	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 <sup>b</sup>	6411.20±255 <sup>a</sup>	5677.20±192.42 <sup>b</sup>	58.57	***
1-42 days	7027.20±161.56 <sup>b</sup>	7911.20±278 <sup>a</sup>	7194.40±298.26 <sup>b</sup>	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 (percentage of BW) of broiler chickens.

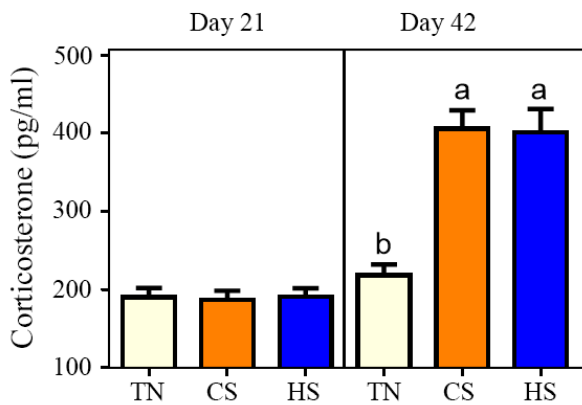
Variables	Treatments			SEM	p
	Thermoneutral	Heat stress	Cold stress		
Antibody titer					
IgG1	2.07±0.20 <sup>a</sup>	1.26±0.10 <sup>b</sup>	1.27±0.10 <sup>b</sup>	0.05	***
IgM1	2.98±0.28 <sup>a</sup>	2.20±0.23 <sup>b</sup>	2.21±0.22 <sup>b</sup>	0.05	***
IgG2	3.54±0.21 <sup>a</sup>	2.65±0.18 <sup>b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

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

of broiler chickens. Nevertheless, although non-significantly, CS had lower negative effects in comparison to HS. Many authors have informed about the adverse effects of CS and HS on the productive performance of poultry (Sahin *et al.*, 2002; Star *et al.*,

2008; Mujahid & Furuse, 2009; Sohail *et al.*, 2012; Fu *et al.*, 2013; Habibian *et al.*, 2014; Tawfeek *et al.*, 2014).

Thermal stress influences the productive performance of poultry by affecting nutrient metabolism and



**Figure 1.** Effects of thermoneutral (TN), cold stress (CS) and heat stress (HS) conditions on corticosterone concentration of broiler chickens on days 21 and 42.

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  $\beta$ -defensin-4, avian  $\beta$ -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 (Campo *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). Shini *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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