Effects of Relative Humidity on Thermoregulation and Hypothalamic Heat Shock Protein 70 Content in Broiler Chickens under Incremental Moderate Heat Stress: Postprint

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Abstract

This experiment aimed to investigate the effects of relative humidity (RH) on thermoregulation and hypothalamic heat shock protein 70 (HSP70) content in broiler chickens under an incremental moderate heat environment. One hundred eighty 21-day-old Arbor Acres (AA) broiler chickens were selected and transferred to an environmental control chamber, randomly divided into 3 groups with 6 replicates per group and 10 chickens per replicate (half male and half female). The preliminary period lasted 7 days at a temperature of 20 °C and RH of 60%. The experimental period lasted 15 days, starting when the chickens were 28 days old. The RH of the three groups was adjusted to 35%, 60%, and 85%, respectively, while the temperature remained at 20 °C. Three days constituted one cycle, for a total of 5 cycles. Beginning from the second cycle, the temperature for all three groups was increased by 3 °C at 10:00 on the first day of each cycle and stabilized within 0.5 h. The temperatures for the five cycles were 20, 23, 26, 29, and 32 °C, respectively. The results showed: 1) The respiratory rate of broilers in the 85% RH group was extremely significantly higher than that in the 60% RH and 35% RH groups ($P<0.01$), and the respiratory rate in the 60% RH group was extremely significantly higher than that in the 35% RH group ($P<0.01$). The core body temperature of broilers in the 85% RH group was significantly higher than that in the 60% RH group ($P<0.05$). The skin temperature of the shank and foot pad in broilers in the 35% RH group was extremely significantly higher than that in the 60% RH and 85% RH groups ($P<0.01$), and the skin temperature of the comb, earlobe, and eyelid in broilers in the 35% RH and 60% RH groups was extremely significantly higher than that in the 85% RH group ($P<0.01$). 2) The serum alkaline phosphatase (AKP) activity of broilers in the 85% RH group was significantly higher than that in

the 35% RH and 60% RH groups (P<0.05). The serum creatine kinase (CK) activity of broilers in the 35% RH and 85% RH groups was significantly higher than that in the 60% RH group (P<0.05). RH had no significant effect on serum aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities in broilers under the incremental moderate heat environment (P>0.05). 3) The serum triiodothyronine (T3) content of broilers in the 85% RH group was significantly higher than that in the 35% RH group (P<0.05). The serum thyroxine (T4) content of broilers in the 35% RH and 85% RH groups was significantly higher than that in the 60% RH group (P<0.05). The serum corticosterone (CORT) content of broilers in the 60% RH and 85% RH groups was extremely significantly higher than that in the 35% RH group (P<0.01). 4) The hypothalamic HSP70 content of broilers in the 35% RH group was significantly lower than that in the 60% RH and 85% RH groups (P<0.05). In conclusion, under the 15-day incremental moderate heat environment of 20–32 °C, high humidity (85%) and low humidity (35%) differentially regulated the thermoregulatory pathways and extent in 28–42-day-old broiler chickens.

Full Text

Effects of Relative Humidity at Gradually Increasing Temperatures on Body Thermoregulation and Hypothalamic Heat Shock Protein 70 Content in Broiler Chickens

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Abstract: This study investigated the effects of relative humidity (RH) on thermoregulation and hypothalamic heat shock protein 70 (HSP70) content in broiler chickens exposed to gradually increasing temperatures. One hundred eighty 21-day-old Arbor Acres (AA) broiler chickens were transferred to environmental control chambers and randomly divided into three groups with six replicates per group and ten birds per replicate (equal numbers of males and females). The pre-trial period lasted 7 days at 20°C and 60% RH. The formal trial lasted 15 days, beginning when the birds were 28 days old. The three groups were maintained at 35%, 60%, and 85% RH respectively, while temperature remained at 20°C. The experiment comprised five consecutive 3-day periods; starting from the second period, the temperature for all three groups was increased by 3°C at 10:00 on the first day of each period, reaching stability within 0.5 hours. The temperatures for the five periods were 20, 23, 26, 29, and 32°C, respectively.

The results showed: (1) The respiratory frequency of broilers in the 85% RH group was significantly higher than that in the 60% and 35% RH groups ($P<0.01$), while the 60% RH group showed significantly higher respiratory frequency than the 35% RH group ($P<0.01$). Core body temperature in the 85% RH group was significantly higher than in the 60% RH group ($P<0.05$). Skin temperatures of the shank and footpad in the 35% RH group were significantly higher than those in the 60% and 85% RH groups ($P<0.01$), while skin temperatures of the comb, earlobe, and eyelid in the 35% and 60% RH groups were significantly higher than in the 85% RH group ($P<0.01$).

(2) Serum alkaline phosphatase (AKP) activity in the 85% RH group was significantly higher than in the 35% and 60% RH groups ($P<0.05$), while serum creatine kinase (CK) activity in both the 85% and 35% RH groups was significantly higher than in the 60% RH group ($P<0.05$). RH showed no significant effect on serum aspartate transaminase (AST) or lactate dehydrogenase (LDH) activities ($P>0.05$).

(3) Serum triiodothyronine (T3) content in the 85% RH group was significantly higher than in the 35% RH group ($P<0.05$). Serum thyroxine (T4) content in both the 35% and 85% RH groups was significantly higher than in the 60% RH group ($P<0.05$). Serum corticosterone (CORT) content in the 60% and 85% RH groups was significantly higher than in the 35% RH group ($P<0.01$).

(4) Hypothalamic HSP70 content in the 35% RH group was significantly lower than in the 60% and 85% RH groups ($P<0.05$). In conclusion, under gradually increasing temperatures from 20 to 32°C over 15 days, high humidity (85%) and low humidity (35%) regulate body heat balance in 28- to 42-day-old broilers through different pathways and to different extents.

Keywords: gradually increasing temperatures; broiler chickens; relative humidity; body thermoregulation; heat shock protein 70

Introduction

Current research on temperature effects in broiler chickens has primarily focused on heat stress environments above 32°C. However, due to the mature application of evaporative cooling pad ventilation systems, extreme heat stress is now relatively uncommon in production settings, whereas moderately hot environments below 32°C frequently occur and similarly adversely affect broiler performance, including growth performance, uncoupling protein expression and glucose-lipid metabolism, intestinal microbial diversity, intestinal immune function, and resting behavior patterns.

Relative humidity (RH) represents a major component of the thermal environment, and its effects on broiler chickens are temperature-dependent. Different

temperature regimes elicit different responses to RH. Studies examining the effects of temperature and RH on broiler growth performance have shown that within thermoneutral and low temperature ranges, RH has no significant effect on daily weight gain. However, at constant temperatures of 32°C or 29°C, high humidity (80%-90%) significantly reduces growth rate compared to low humidity (40%). When temperature is suddenly increased to 35°C, 28°C, or 30°C with RH at 60%-65%, broilers exhibit the highest growth rate and feed intake.

When confronted with adverse environmental conditions, poultry initiate a series of regulatory responses to maintain dynamic body heat balance, involving physiological and biochemical reactions related to thermoregulation, including body temperature, respiration, and endocrine responses. Heat shock proteins (HSPs) are proteins produced in response to stressor stimuli and represent the primary hallmark of the heat shock response in organisms. Among the HSP family, HSP70 is the most important member, and its broad biological functions has made it a major focus in life sciences research.

In most regions of China, temperatures increase gradually during the spring-summer transition, yet the effects of RH on thermoregulation and hypothalamic HSP70 content in broilers under gradually increasing temperatures remain unreported. Therefore, this study investigated the influence of RH on thermoregulation and hypothalamic HSP70 content in broilers exposed to gradually increasing temperatures, aiming to provide a scientific basis for determining appropriate RH levels in poultry production.

1.1 Experimental Design

One hundred eighty 21-day-old Arbor Acres (AA) broiler chickens from the same hatch batch, with consistent management and good health status, were transferred to environmental control chambers and randomly divided into three groups with six replicates per group and ten birds per replicate (equal numbers of males and females). The pre-trial period lasted 7 days at 20°C and 60% RH. During the 15-day formal trial beginning at 28 days of age, the three groups were maintained at 35%, 60%, and 85% RH, respectively, while temperature remained at 20°C. The experiment comprised five consecutive 3-day periods; starting from the second period, the temperature for all groups was increased by 3°C at 10:00 on the first day of each period, reaching stability within 0.5 hours. The temperatures for the five periods were 20, 23, 26, 29, and 32°C, respectively.

1.2 Experimental Diets

A corn-soybean meal diet was formulated according to NRC (1994) nutrient requirements. The basal diet composition and nutrient levels are presented in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (as-fed basis), %

Items	Content
Ingredients	
Corn	
Soybean meal	
Soybean oil	
NaCl	
Limestone	
$CaHPO_4$	
DL-Met	
Premix[1]	
Total	
Nutrient levels[2]	
ME (MJ/kg)	
CP	
Ca	
AP	
Lys	
Met	
Met+Cys	

[1]Premix provided the following per kg of diet: VA 10,000 IU, VD_3 3,400 IU, VE 16 IU, VK_3 2.0 mg, VB_1 2.0 mg, VB_2 6.4 mg, VB_6 2.0 mg, VB_{12} 0.012 mg, pantothenic acid calcium 10 mg, nicotinic acid 26 mg, folic acid 1 mg, biotin 0.1 mg, choline 500 mg, Zn ($ZnSO_4 \cdot 7H_2O$) 40 mg, Fe ($FeSO_4 \cdot 7H_2O$) 80 mg, Cu ($CuSO_4 \cdot 5H_2O$) 8 mg, Mn ($MnSO_4 \cdot H_2O$) 80 mg, I (KI) 0.35 mg, Se (Na_2SeO_3) 0.15 mg.

[2]Nutrient levels were calculated values.

1.3 Management Practices

All experimental birds were floor-reared using single-tier floor cages developed by our laboratory, with a stocking density of 0.64 m^2 per 8 birds. Birds had ad libitum access to feed and water, 24-hour lighting, and routine immunization throughout the trial period.

1.4 Measurements

1.4.1 Physiological Indices On day 15 of the formal trial (48 hours after temperature reached 32°C), skin temperature, core body temperature, and respiratory frequency were measured. Skin temperature was measured using an infrared thermal imager (FLIR E4, thermal resolution 0.07°C, accuracy $±$2%). The lateral side of the head and shank (metatarsal) region were photographed

vertically from 0.5 m distance at 3-minute intervals for 1 hour, yielding 20 infrared images per bird. Skin temperatures of the comb, shank, footpad, eyelid, and earlobe were analyzed using FLIR Tools software, and the average of 20 measurements per bird was used as the true skin temperature. Core body temperature was measured by inserting a 5 cm probe of a digital thermometer (Model JM 6200, resolution 0.01°C) almost completely into the rectum of six randomly selected birds per group (one per replicate, equal sex ratio). After stabilization, values were recorded at 5-second intervals four times, and the average was calculated. Respiratory frequency was measured in six randomly selected birds per group (one per replicate, equal sex ratio) by counting breaths per minute at 10-minute intervals for six total measurements, with the average representing respiratory frequency.

1.4.2 Serum Enzyme Activities and Endocrine Indices On day 15 of the formal trial (48 hours after temperature reached 32°C), blood samples were collected from the wing vein of six randomly selected birds per group (one per replicate, equal sex ratio). Serum was separated by centrifugation at 3,000 r/min for 10 minutes and stored at -80°C. Serum activities of aspartate transaminase (AST), alkaline phosphatase (AKP), lactate dehydrogenase (LDH), and creatine kinase (CK), as well as contents of triiodothyronine (T3), thyroxine (T4), and corticosterone (CORT) were determined by enzyme-linked immunosorbent assay (ELISA) using commercial kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

1.4.3 Hypothalamic HSP70 Content On day 15 of the formal trial (48 hours after temperature reached 32°C), six birds per group (one per replicate, equal sex ratio) were sacrificed immediately after blood collection. The hypothalamus was dissected within 3 minutes, placed in cryovials, and stored at -80°C. Hypothalamic HSP70 content was determined by double-antibody sandwich ELISA.

1.5 Statistical Analysis

Data were analyzed using one-way ANOVA in SAS 9.2 software. Duncan' s multiple range test was used for post-hoc comparisons. Results are expressed as means ± standard deviation. Differences were considered significant at $P<0.05$ and highly significant at $P<0.01$.

Results

2.1 Effects of RH on Physiological Indices of Broilers Under Gradually Increasing Temperatures

As shown in Table 2 , RH had highly significant effects on respiratory frequency and skin temperature of broilers under gradually increasing temperatures from

20 to 32°C over 15 days (P<0.01). Respiratory frequency in the 85% RH group was significantly higher than in the 60% and 35% RH groups (P<0.01), while the 60% RH group showed significantly higher respiratory frequency than the 35% RH group (P<0.01). Core body temperature in the 85% RH group was significantly higher than in the 60% RH group (P<0.05), with no significant difference between the 35% RH group and the other two groups (P>0.05). Shank and footpad skin temperatures in the 35% RH group were significantly higher than those in the 60% and 85% RH groups (P<0.01), while comb, earlobe, and eyelid skin temperatures in the 35% and 60% RH groups were significantly higher than in the 85% RH group (P<0.01).

Table 2 Effects of relative humidity at gradually increasing temperatures on physiological indices of broiler chickens

Items	Relative humidity (%)		P-value
	35	60 85	
Respiratory frequency (breaths/min)	93.44$±20.04^{cc}\|112.00±24.35^{BB}\|130.28±10.32^{AA}\| < 0.0001\|\|$Corebodytemperature(°C)$\|42.55±0.29^{ab}\|42.10±0.24^{b}\|42.61±0.52^{a}\|\|\|∗ ∗$Skintemperature(°C)$ ∗ ∗|||||Leg|44.51±0.60^{AA}|43.50±0.51^{BB}|42.49±1.81^{cc}| < 0.0001||$Flipper$|43.66±0.61^{AA}|42.46±0.93^{BB}|42.07±1.85^{BB}| < 0.0001||$Earlobe$|42.82±0.60^{AA}|42.95±0.82^{AA}|40.30±1.74^{BB}| < 0.0001||$Comb$|41.76±0.57^{AA}|41.87±0.69^{AA}|39.25±1.59^{BB}| < 0.0001||$Eyelid$|42.58±0.52^{AA}|42.40±1.02^{AA}|40.07±$1.74	

In the same row, values with different lowercase superscripts differ significantly (P<0.05), different capital superscripts differ highly significantly (P<0.01), and same or no superscripts indicate no significant difference (P>0.05). The same applies below.

2.2 Effects of RH on Serum Enzyme Activities of Broilers Under Gradually Increasing Temperatures

As shown in Table 3 , RH significantly affected serum AKP and CK activities in broilers under gradually increasing temperatures from 20 to 32°C over 15 days (P<0.05). Serum AKP activity in the 85% RH group was significantly higher than in the 35% and 60% RH groups (P<0.05), with no significant difference between the latter two groups (P>0.05). Serum CK activity in both the 35% and 85% RH groups was significantly higher than in the 60% RH group (P<0.05), with no significant difference between the 35% and 85% RH groups (P>0.05). RH showed no significant effect on serum AST or LDH activities (P>0.05).

Table 3 Effects of relative humidity at gradually increasing temperatures on serum enzyme activities of broiler chickens

Items	Relative humidity (%)			P-value
	35	60	85	
AST (U/L)	67.69$±15.40\|58.55±10.73\|53.93±11.72\|\|\|$AKP(U/L)$\|101.72±52.14^{b}\|146.69±76.81^{b}|312.72±202.4			

2.3 Effects of RH on Endocrine Indices of Broilers Under Gradually Increasing Temperatures

As shown in Table 4 , RH significantly affected serum T3, T4, and CORT contents in broilers under gradually increasing temperatures from 20 to 32°C over 15 days (P<0.05). Serum T3 content in the 85% RH group was significantly higher than in the 35% RH group (P<0.05), with no significant difference between the 60% RH group and the other two groups (P>0.05). Serum T4 content in both the 35% and 85% RH groups was significantly higher than in the 60% RH group (P<0.05), with no significant difference between the 35% and 85% RH groups (P>0.05). Serum CORT content in the 60% and 85% RH groups was significantly higher than in the 35% RH group (P<0.01), with no significant difference between the 60% and 85% RH groups (P>0.05).

Table 4 Effects of relative humidity at gradually increasing temperatures on endocrine indices of broiler chickens

Items	Relative humidity (%)			P-value
	35	60	85	
T3 (ng/mL)	138.56$±18.83^{b}\|155.03±24.38^{ab}\|164.97±10.72^{a}\|\|\|$T4(\mu g/L)$\|164.96±14.89^{a}\|142.36±21.78^{b}|160.18±			

2.4 Effects of RH on Hypothalamic HSP70 Content of Broilers Under Gradually Increasing Temperatures

As shown in Figure 1 [Figure 1: see original paper], RH significantly affected hypothalamic HSP70 content in broilers under gradually increasing temperatures from 20 to 32°C over 15 days (P<0.05). Hypothalamic HSP70 content in the 35% RH group was significantly lower than in the 60% and 85% RH groups (P<0.05), with no significant difference between the latter two groups (P>0.05).

Figure 1 Effects of relative humidity at gradually increasing temperatures on hypothalamic HSP70 content in broiler chickens

Columns with different lowercase letters differ significantly (P<0.05), while columns with the same letters indicate no significant difference (P>0.05).

Discussion

3.1 Effects of RH on Physiological Indices of Broilers Under Gradually Increasing Temperatures

Core body temperature and respiratory frequency are important physiological indicators reflecting thermoregulatory responses in broiler chickens. Yahav measured cloacal and skin temperatures (featherless area under the wing) and reported that at a constant 28°C ambient temperature, RH (40%-45%, 50%-55%, 60%-65%, and 70%-75%) had no significant effect on body temperature of 4- to 8-week-old broilers. Lin measured skin temperature (chest, back, toe, leg, and wing regions) and cloacal temperature using an infrared point thermometer and thermistor probe, respectively, and found that RH had no significant effect on broiler body temperature when ambient temperature was below 25°C.

Infrared thermography offers numerous advantages including high precision, non-contact measurement, non-invasiveness, and minimal disturbance to subjects, ensuring objective and reliable data. This technology has been increasingly applied in poultry skin temperature measurement. Our study found that under gradually increasing temperatures from 20 to 32°C over 15 days, broilers exhibited higher skin temperatures but lower respiratory frequency under low humidity (35%), whereas high humidity (85%) resulted in lower skin temperatures but higher respiratory frequency. Under high humidity, the high moisture content in the environment increases water content on the skin surface, hindering evaporative heat loss. Consequently, broilers must accelerate respiratory evaporation for heat dissipation, but excessive respiration places them under high metabolic load, compromising thermoregulation and elevating core body temperature. Conversely, under low humidity, exposed skin areas (particularly legs) showed higher temperatures while respiratory frequency remained low, likely because low humidity facilitates skin evaporation from exposed areas while limiting respiratory evaporative heat loss. These findings indicate that under gradually increasing temperatures from 20 to 32°C over 15 days, broilers primarily rely on cutaneous evaporative heat loss to maintain thermal balance under low humidity, whereas under high humidity they depend mainly on respiratory evaporative heat loss, though excessive respiration increases metabolic load and elevates core body temperature.

3.2 Effects of RH on Serum Enzyme Activities of Broilers Under Gradually Increasing Temperatures

Lactate dehydrogenase (LDH) is a key enzyme in glycolysis that catalyzes pyruvate conversion to lactate, and elevated serum LDH activity is closely associated with enhanced anaerobic glycolysis. Under normal conditions, aspartate transaminase (AST) resides in mitochondria of cardiac and hepatic cells, with minimal activity in serum; during stress, mitochondrial damage causes AST to leak into the bloodstream, increasing serum AST activity. Elevated serum AST activity indicates hepatocellular injury. Our study found that RH had no signif-

icant effect on LDH or AST activities under gradually increasing temperatures from 20 to 32°C over 15 days, suggesting that RH stress under these conditions does not enhance anaerobic glycolysis or cause hepatocellular damage.

Alkaline phosphatase (AKP) comprises a group of enzymes with high activity under alkaline conditions and plays an important role in protein and lipid catabolism. During stress, animals mobilize glycogen, protein, and lipid reserves from body stores (muscle) for energy, resulting in increased AKP activity. Our study demonstrated that RH significantly affected AKP activity, with the high humidity (85%) group showing significantly higher serum AKP activity than the low (35%) and moderate (60%) humidity groups. This indicates that high humidity imposes greater stress, requiring mobilization of glycogen, protein, and lipid reserves for energy, consequently increasing AKP activity.

Creatine kinase (CK), primarily composed of muscle isoforms (MM-CK), serves as an important indicator of stress in animals. Sandercock et al. reported that exposure to 32°C and 75% RH for 2 hours significantly increased plasma CK activity in broilers, indicating that elevated blood CK activity reflects stress responses. Our study found that under gradually increasing temperatures from 20 to 32°C over 15 days, both high (85%) and low (35%) humidity significantly increased serum CK activity compared to moderate humidity (60%). This may be because stress from both low and high humidity reduces feed intake and nutrient intake, leading to muscle malnutrition, compromised muscle cell membrane function and permeability, and leakage of muscle CK into the bloodstream, thereby increasing serum CK activity.

3.3 Effects of RH on Endocrine Indices of Broilers Under Gradually Increasing Temperatures

The thyroid gland is a crucial endocrine organ regulating metabolism. Thyroid hormones T3 and T4 play important roles in energy metabolism and thermogenesis, serving as common stress indicators in animals. Yahav et al. found that at temperatures of 28°C, 30°C, or 35°C with RH of 40%-75%, serum T3 content was positively correlated with feed intake in broilers. Other reports indicate that under high temperature and humidity conditions, reduced heat dissipation capacity necessitates decreased heat production through endocrine pathways to maintain thermal balance, and declining plasma T3 content reflects this regulatory mechanism and indicates enhanced heat tolerance. However, our study found that under gradually increasing temperatures from 20 to 32°C over 15 days, serum T3 content in the high humidity (85%) group was significantly higher than in the low humidity (35%) group, while serum T4 content in both high (85%) and low (35%) humidity groups was significantly higher than in the moderate humidity (60%) group. These discrepancies may be related to our experimental conditions—a chronic, long-term, gradually increasing heat stress over 15 days—whereas previous studies involved different stress patterns. Additionally, research has shown that blood T3 and T4 contents fluctuate inconsistently during heat stress depending on duration.

Corticosterone (CORT), the primary glucocorticoid in poultry, participates in mobilizing stored energy reserves to prepare animals for adverse stress. Generally, stress stimulates the hypothalamic-pituitary-adrenal axis to secrete glucocorticoids, increasing CORT content to resist stress. Our study found that under gradually increasing temperatures from 20 to 32°C over 15 days, serum CORT content in the low humidity (35%) group was significantly lower than in the moderate (60%) and high (85%) humidity groups. This may be because low humidity conditions primarily facilitate cutaneous evaporative heat loss—a physical cooling mechanism that does not consume internal energy reserves, resulting in less stress and consequently lower serum CORT content. The specific molecular mechanisms underlying this response require further investigation.

3.4 Effects of RH on Hypothalamic HSP70 Content of Broilers Under Gradually Increasing Temperatures

Heat shock proteins were first discovered by Ritossa et al. in 1962 in Drosophila salivary gland chromosomes exposed to high temperature. HSPs are proteins produced by cells in response to stressors, particularly heat, with the HSP70 family being the most important and evolutionarily conserved. During stress, organisms enter an oxidative stress state with increased reactive oxygen species (ROS) production, and HSPs are generated and released to protect cells from ROS damage. Xie et al. found that both acute and chronic heat stress upregulated HSP expression in heart, liver, and muscle tissues of laying hens. Similarly, Xu reported upregulated HSP gene expression in chickens under heat stress. Numerous studies have demonstrated a positive correlation between HSP production and heat tolerance.

Our study found that under gradually increasing temperatures from 20 to 32°C over 15 days, 35% RH significantly reduced hypothalamic HSP70 content. This may be because low humidity conditions primarily enable cutaneous evaporative heat loss—a physical cooling mechanism that does not consume internal energy, resulting in lower ROS production and consequently lower HSP70 content. Additionally, intracellular free calcium and other factors are involved in HSP70 transcriptional regulation, though the specific molecular mechanisms require further investigation.

Conclusion

Under gradually increasing temperatures from 20 to 32°C over 15 days, high humidity (85%) significantly increased respiratory frequency, core body temperature, serum AKP and CK activities, and T4 content while significantly decreasing skin temperature (except footpad temperature). Low humidity (35%) significantly increased shank and footpad temperatures, serum CK activity, and T4 content while significantly decreasing respiratory frequency, serum CORT, and hypothalamic HSP70 content. These findings demonstrate that high and low humidity regulate body heat balance through different pathways and to

different extents in 28- to 42-day-old broilers under gradually increasing temperatures.

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Note: Figure translations are in progress. See original paper for figures.