Effects of Different Relative Humidity on Physiological Indicators of Broiler Chickens Under Continuous Daily Temperature Increase: Postprint

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Abstract

This study investigated the effects of different relative humidity (RH) levels on physiological indicators of broiler chickens in a daily warming environment. One hundred eighty Arbor Acres (AA) broiler cockerels at 22 days of age were selected and transferred to an artificial climate chamber, randomly divided into 3 groups with 6 replicates per group and 10 birds per replicate. The acclimation period lasted 7 days at a temperature of 20 °C and RH of 60%. At 29 days of age, the experimental RH levels were adjusted to 35%, 60%, and 85% respectively until the end of the experiment. The temperature in the environmental control chamber was increased by 2 °C daily, with a total experimental period of 9 days. At 37 days of age, the chamber temperature reached 38 °C; during the experimental period, broilers had ad libitum access to feed and water, and no birds were handled throughout the trial. Non-contact physiological indicators were selected for measurement to minimize stress responses in the broilers. The results showed that broilers exhibited inflection point temperatures (IPt) for core temperature (CT), ear lobe temperature (ET), shank temperature (LT), respiration rate (RR), feed intake (FI), and water intake (DW). The first parameter to change was LT, with the ambient temperature range for change being 24.6–25.1 °C, followed by ET and CT, with temperature ranges of 25.0-25.1 °C and 25.5-26.4 °C, respectively. RR exhibited two IPts (IPt1 and IPt2), with temperature ranges of 25.5–27.2 °C and 32.5–33.3 °C, respectively. When ambient temperature exceeded the IPt1 value, RR increased with rising temperature; when ambient temperature exceeded the IPt2 value, RR decreased with rising temperature. The ambient temperature ranges for changes in FI and DW were 27.1-29.3 °C and 29.2–29.5 °C, respectively; when ambient temperature exceeded the IPt values, both FI and DW decreased with rising temperature. The IPt value for FI in the high humidity group was significantly higher than that in the low and moderate humidity groups ($P < 0.05$), while the IPt2 value for mortality rate (MR) in the high humidity group was significantly lower than that in the low and moderate humidity groups ($P < 0.05$). The IPt values for CT, ET, LT, RR, FI, and DW in the high humidity group were lower than those in the moderate and low humidity groups, though RH had no significant effect on the model parameters for ET, LT, RR, and DW ($P > 0.05$). The results suggest that ambient temperature had a greater influence on broiler physiological indicators, while RH had a lesser effect. The IPt values for FI decline and CT increase can be considered as the upper critical temperature; the upper critical temperature for CT in 4-5-week-old broiler cockerels was 25.5-26.4 °C; the upper critical temperatures for the onset of increased RR and decreased FI were 25.5-27.2 °C and 27.1-29.2 °C, respectively; the upper critical temperature for mortality was 32.7-33.5 °C. At the time of death, CT in broilers was 4.6-5.1 °C higher than the normal value. Different physiological indicators in 4-5-week-old broiler cockerels had different upper critical temperatures.

Full Text

Effects of Different Relative Humidity on Physiological Indices of Broilers under Continuous Daily Temperature Increase

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Abstract

This study investigated the effects of different relative humidity (RH) levels on physiological indices of broiler chickens under a daily incremental temperature regimen. One hundred eighty 22-day-old Arbor Acres (AA) male broilers were transferred to environmental chambers and randomly divided into 3 groups with 6 replicates per group and 10 birds per replicate. An adaptation period lasted 7 days at 20 °C and 60% RH. At 29 days of age, RH was adjusted to 35%, 60%, and 85% for the respective groups until trial completion. Chamber temperature increased by 2 °C daily over a 9-day period, reaching 38 °C when birds were 37 days old. Broilers had ad libitum access to feed and water throughout the experiment. To minimize stress, all physiological measurements were conducted using non-contact methods without handling the birds.

The results revealed inflection point temperatures (IPt) for core temperature (CT), earlobe temperature (ET), leg temperature (LT), respiratory rate (RR), feed intake (FI), and drinking water consumption (DW). LT responded first, with changes occurring between 24.6–25.1 °C, followed by ET (25.0–25.1 °C) and CT (25.5–26.4 °C). RR exhibited two IPts (IPt$_1$ and IPt$_2$) at 25.5–27.2 °C and 32.5–33.3 °C, respectively: RR increased with temperature above IPt$_1$ but decreased above IPt$_2$. FI and DW began changing at 27.1–29.3 °C and 29.2–29.5 °C, respectively, declining when temperature exceeded their IPt values. The IPt for FI in the high-humidity group was significantly lower than in low- and medium-humidity groups ($P < 0.05$), while the IPt$_2$ for mortality rate (MR) was significantly lower in the high-humidity group ($P < 0.05$). Although RH did not significantly affect model parameters for ET, LT, RR, or DW ($P > 0.05$), all IPt values for CT, ET, LT, RR, FI, and DW were lower in the high-humidity group compared to medium- and low-humidity groups.

These findings indicate that ambient temperature strongly influences broiler physiological indices, whereas RH has relatively minor effects. The IPt values where FI declines and CT rises can be considered upper critical temperatures. For 4–5-week-old male broilers, upper critical temperatures were 25.5–26.4 °C for CT, 25.5–27.2 °C for RR elevation, 27.1–29.2 °C for FI reduction, and 32.7–33.5 °C for mortality. At death, CT exceeded normal values by 4.6–5.1 °C. Different physiological indices in 4–5-week-old male broilers have distinct upper critical temperatures.

Keywords: broiler; humidity; increasing temperature; inflection point temperature

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Introduction

Modern fast-growing white-feathered broilers exhibit rapid growth, high feed conversion efficiency, and low feed-to-weight ratios, with breast and leg muscles comprising over 90% of total muscle mass. Rapid breast muscle growth increases heat production, yet broilers possess underdeveloped heat dissipation systems, making them susceptible to environmental stimuli and reducing stress tolerance. Elevated ambient temperature increases core temperature (CT), leg temperature (LT), earlobe temperature (ET), and respiratory rate (RR) while decreasing feed intake (FI). Previous studies have shown that RH below 25 °C does not affect broiler surface temperature, and at 28 °C, RH has no significant effect on CT or surface temperature. However, at 35 °C ambient temperature, high RH significantly increases rectal, back, and abdominal temperatures, as high humidity inhibits evaporative heat dissipation in poultry. When RH exceeds the balance point between heat production and dissipation, respiratory evaporative cooling becomes insufficient to maintain CT homeostasis, leading to hyperthermia.

Most previous studies employed constant temperature regimens, whereas in production environments, temperature fluctuates, particularly during spring-summer transitions with daily temperature increases. The effects of RH on broiler physiological indices under incremental temperature conditions remain unreported. Therefore, this study investigated the effects of different RH levels on non-contact physiological indices of broilers under daily temperature increase to determine upper critical temperatures for various parameters at different humidity levels, providing a basis for graded temperature-humidity management in broiler production.

1.1 Experimental Animals and Design

One hundred eighty 22-day-old Arbor Acres (AA) male broilers with uniform hatching, management, and body weight were randomly divided into 3 groups with 6 replicates per group and 10 birds per replicate. Birds were housed in three environmental chambers during a 7-day adaptation period at 20 °C and 60% RH. At 29 days of age, the experimental period began with RH set at 35%, 60%, and 85% for the respective groups, maintained constant until trial completion. Chamber temperature increased by 2 °C daily at 09:00, with the increase completed within 1 hour. The experimental period lasted 9 days.

1.2 Management

The experiment was conducted in environmental chambers with automatic temperature and humidity control and 24-hour lighting. Single-tier cage systems developed by our laboratory were used, with a stocking density of 10 birds per 0.8 m^2. The basal diet was formulated according to NRC (1994) standards, with composition and nutrient levels shown in Table 1 . Birds had ad libitum access to feed and water. To minimize stress, all measurements were conducted without handling the birds, and unauthorized personnel were prohibited from entering the chambers.

Feed and water were provided at 08:00 and 20:00 daily from the start of the experiment at 29 days of age. Total feed weight and residual feed were recorded daily, along with water additions, to calculate average daily FI and DW per bird per replicate for regression modeling.

1.3.2 Measurement of CT, Surface Temperature, and RR

Daily at 14:30, infrared thermal imaging was used to capture lateral head and leg images from 5 cm perpendicular distance. Ten images were taken per replicate per body part, with random bird selection, to determine ET and LT using software analysis. RR measurement began at 15:30 by counting breaths over 30 seconds for 6 randomly selected birds per replicate. During later stages when RR became too rapid for visual counting, video recording was used for subsequent analysis. Core temperature was recorded using micro-temperature loggers (DS1922L, USA, accuracy $±$0.5 °C), calibrated using a standard mercury thermometer certified by the Haidian District Metrology and Testing Institute of Beijing, following Purswell et al. Loggers were programmed to record every 30 minutes. Two birds per replicate were marked with blue dye on feathered areas;

at 28 days of age, these marked birds were administered loggers orally to reside in the crop until death or trial completion.

1.3.3 Mortality Rate Measurement

Daily mortality counts and time of death were recorded throughout the experiment.

1.4 Data and Statistical Analysis

Data were analyzed using SPSS 17.0 statistical software with nonlinear regression analysis. The nonlinear regression model was:

$$Y = C \times RH + K \times RH \times (T - IPt \times RH) \quad (T \geq IPt \times RH)$$

$$Y = C \quad (T < IPt \times RH)$$

Where Y represents the dependent variable changing with ambient temperature (CT, ET, LT, RR, FI, DW, or MR), C is the constant value of Y before change at different RH levels, K is the slope after change, T is ambient temperature, and IPt is the inflection point temperature where Y begins to change. Models with higher regression coefficients were selected when trend lines differed.

2.1 Effects of Different RH on CT, Surface Temperature, and RR Under Continuous Temperature Increase

CT, ET, and LT followed broken-line models (Table 2), remaining constant below IPt and increasing linearly above it. To exclude abnormal CT values during the 2-hour period preceding death and reduce error, these data were removed before regression analysis.

Table 2 Model parameters of CT, ET, and LT

Item	RH (%)	Constant C (°C)	IPt (°C)	Slope K
CT	35	41.17\$±0.08|25.55±1.34|0.25±0.04^{a}|||60|40.99±0.28|26.38±1.89|0.29±0.07^{ab}|||85|41.03		

In the same column, values with no letter or the same letter superscripts indicate no significant difference (P > 0.05), while different letters indicate significant difference (P < 0.05). The same applies below.

Nonlinear regression curves for CT, ET, and LT are shown in Figures 1 [Figure 1: see original paper], 2 [Figure 2: see original paper], and 3 [Figure 3: see original paper], respectively.

RR model parameters are presented in Table 3 . RR exhibited two IPts: IPt_1 where RR reached maximum values, and IPt_2 where RR began to decline. RH had no significant effect on RR model parameters ($P > 0.05$).

Table 3 Model parameters of RR

Item	Constant C (breaths/min)	Slope K (breaths/min/°C)	IPt_1 (°C)	IPt_2 (°C)
RR	20.98\$±1.19|12.39±5.04|27.15±1.45|32.58±0.93|||19.45±1.78|8.72±2.99|26.74±0.65|33.25±1.80|||21.81±			
P-value				> 0.05

The nonlinear regression curve for RR is shown in Figure 4 [Figure 4: see original paper].

2.2 Effects of Different RH on FI and DW Under Continuous Temperature Increase

Model parameters for FI and DW are shown in Table 4 . FI remained stable below its IPt and declined above it. DW increased with temperature until reaching its IPt, then decreased. The IPt for FI in the high-humidity group was significantly lower than in low- and medium-humidity groups ($P < 0.05$).

Table 4 Model parameters of FI and DW

Item	Constant C (g)	IPt (°C)	Slope 1 K_1 (g/°C)	Slope 2 K_2 (g/°C)
FI	166.92\$±3.68|29.08±3.68^b|−15.49±1.18||||174.04±5.61|29.18±5.61^b|−14.86±1.20||||173.07±6.76|27.07±6.76^a|−13.55±5.43|||*DW*|29.30±0.59|15.88±4.17|−43.64±4.46||||29.50±0.73|15.36±3.20|−42.35±6.38||||29.20±0.41|15.69±3.85|−55.54±\$10.77			
P-value	< 0.01			

Regression curves for FI and DW are shown in Figures 5 [Figure 5: see original paper] and 6 [Figure 6: see original paper]. Before the inflection point, FI ranked as medium > high > low humidity groups; after the inflection point, the ranking was medium > low > high humidity groups. Before the DW inflection point, values increased from low to medium to high humidity groups.

2.3 Effects of Different RH on MR Under Continuous Temperature Increase

MR model parameters are presented in Table 5 . Two IPts were identified: mortality began at IPt_1 and increased linearly, reaching 100% at IPt_2. The high-humidity group exhibited the earliest mortality and fastest death rate, with IPt_2 significantly lower than in medium- and low-humidity groups ($P < 0.05$).

Table 5 Model parameter values of MR

<table>
<tr><th>Item</th><th>IPt_1 (°C)</th><th>Slope K (%/°C)</th><th>IPt_2 (°C)</th></tr>
<tr><td>MR</td><td colspan="3">18$±0.03|33.48±0.44|38.92±1.20^{a}|||21±0.04|33.54±0.78|38.51±0.27^{a}|||26±0.06|32.72±0.88|36.84±</td></tr>
<tr><td>P-value</td><td></td><td></td><td>< 0.01</td></tr>
</table>

The MR curve is shown in Figure 7 [Figure 7: see original paper]. Table 6 shows the average difference between maximum CT at death and the regression constant, which was not significantly affected by RH ($P > 0.05$).

Table 6 Average differences between the highest CT value at death and constant values

<table>
<tr><th>Differences (°C)</th><th>P-value</th></tr>
<tr><td colspan="2">4.61$±0.59| > 0.05||5.05±0.59|||5.03±$0.52</td></tr>
</table>

Overall results indicate that IPt values for CT, ET, LT, RR, FI, DW, and MR were all lower in the high-humidity group compared to medium- and low-humidity groups.

Discussion

The thermoneutral zone for adult poultry is 18–22 °C, where heat production and dissipation are minimal and birds maintain comfort. When ambient temperature exceeds this zone, broilers initiate thermoregulation by increasing surface temperature and altering behavior. As temperature rises further, poultry increase RR for heat dissipation. If temperature continues to increase beyond the capacity of respiratory cooling to balance heat production, broilers cannot maintain normal body temperature and must reduce FI to decrease heat production while increasing DW to prevent further CT elevation.

Previous research reported that 26 °C induces mild heat stress without changing CT, while 30 °C significantly elevates CT. At constant 30 °C, high humidity (80%) significantly increased comb, wing, shank, and toe temperatures compared to low humidity (40%), along with rectal and breast temperatures. Another study found no significant difference in rectal and skin temperatures between 30 °C and 20 °C, but rectal temperature increased by 1.5 °C at 40 °C compared to 30 °C, with back, leg, and wing temperatures rising progressively. Cyclic high temperature (28-32 °C) increased rectal temperature by 1.65 °C and RR by 3.7-fold compared to constant 23 °C.

Our results show LT reached its IPt first (24.6–25.7 °C), followed by ET (24.9-25.1 °C) and CT (25.5-26.4 °C), with no significant RH effects on C values, K values, or IPts. Below IPt, medium-humidity group C values for ET, LT, and CT were lower than low- and high-humidity groups, though not significantly different. Above 27.14 °C, CT was highest in the high-humidity group, which also had higher LT than other groups. The earlier CT inflection compared to previous studies reflects modern broilers' enhanced sensitivity to heat due to selection for rapid muscle growth without corresponding improvement in thermoregulatory mechanisms.

During heat stress, animals primarily dissipate heat through evaporation. Lacking sweat glands, poultry increase respiratory evaporation to maintain CT. Thermoregulatory failure leads to CT elevation. In this study, CT, LT, and ET reached upper critical temperatures around 25 °C while RR had not yet changed significantly, likely due to modern broilers' heightened heat sensitivity. Although RR tended to increase after exceeding the thermoneutral zone, the elevation was small and non-significant, while CT increased more markedly and reached significance earlier, resulting in a lower IPt for CT than for RR.

The upper critical temperature for FI was 27.1-29.2 °C. Previous studies reported no significant RH effect on FI in 4-8 week-old broilers at 23.8 °C, while constant 35 °C decreased FI by 13% compared to 20 °C. Another study found FI decreased by 8 g at 31 °C versus 26 °C. In our study, FI decreased by 15.5, 14.9, and 13.6 g per 1 °C increase above IPt at 35%, 60%, and 85% RH, respectively. Differences may arise because constant heat exposure allows longer acclimation, whereas daily temperature increases impose continuous novel stress requiring constant adjustment. The significantly lower FI IPt in the high-humidity group suggests that while broilers increase RR for cooling, they must also reduce heat production. Since feed is the primary energy source, reducing FI decreases body heat but also reduces energy and nutrients needed for bodily functions.

Studies have shown that constant 32 °C significantly increased DW compared to 22 °C, while another reported DW increased significantly during the first week but decreased during the second week at constant 30 °C versus 21 °C. Our DW upper critical temperature was 29.2-29.3 °C. Below IPt, DW increased with temperature as broilers increased respiratory evaporative water loss to maintain CT, consistent with most studies. Above IPt, DW decreased, possibly because reduced FI led to lower daily gain and malnutrition. In hot environments, broilers reduce activity and basal metabolic rate to decrease total heat production, and the energy cost of accessing water may lead them to forego drinking. Additionally, water temperature in chamber-located drinkers increased with ambient temperature, contributing to reduced DW. While RH did not affect DW model parameters, lower humidity tended to increase DW below IPt, though not significantly.

Heat stress significantly elevates RR, with reports of RR increasing to 100 breaths/min within 3 hours at 30 °C and from 9.5 to 94 breaths/min at 35 °C, followed by a decline when temperature increased further to 36.8 °C after 4 hours. Our results show RR increased at rates of 12.4, 8.7, and 9.1 breaths/min per °C between 27.2-32.5 °C at 35%, 60%, and 85% RH, respectively, indicating low humidity enhanced respiratory evaporative cooling. Maximum RR reached 76-88 breaths/min. The decline in RR above 32.5-33.3 °C may result from malnutrition, declining physiological function, and the energy cost of respiration itself, ultimately leading to mortality.

The upper critical temperature for mortality was 32.7-33.5 °C in this daily incremental temperature regimen, with the lethal limit reached at 36.8-39.0 °C. The significantly lower IPt_2 for MR in the high-humidity group demonstrates that higher humidity accelerates mortality. Previous studies reported that CT at death increased by 4.59 °C in heat tolerance tests. Our results show that when CT exceeded constant values by approximately 4-6 °C, broilers reached a critical life-threatening state. Although the maximum temperature in this study (38 °C) did not exceed broiler body temperature, the heightened heat sensitivity of modern broilers necessitates special attention in production settings.

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