

## Effects of Relative Humidity on Growth Performance, Behavior, and Jejunal Morphology of Broiler Chickens Under Intermittent Mild Heat Stress: Postprint

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### Abstract

This experiment aimed to investigate the effects of relative humidity (RH) on growth performance, behavior, and jejunal morphology of broilers under a moderately hot environment of 31 °C. A total of 180 22-day-old Arbor Acres broilers were randomly divided into 3 groups (Group , Group , and Group ) and transferred to environmental control chambers, with 6 replicates per group and 10 birds per replicate (half male and half female). There was a 7-day preliminary period, during which the chamber temperature was 21 °C and RH was 60%. The formal experimental period lasted 14 days. Starting from 29 days of age, the ambient temperature in the chicken house was maintained at a constant 31 °C daily from 10:00 to 16:00 (6 h). The RH for Groups , , and was 30%, 60%, and 85%, respectively. The environmental temperature and humidity during the remaining time were the same as during the preliminary period. The results showed: 1) On days 1-14, the average daily feed intake (ADFI), average daily gain (ADG), and average daily water intake of Group were significantly lower than those of Group ( $P<0.05$ ). 2) On days 1, 7, and 14 of the experiment, the proportion of resting behavior frequency in Group was significantly lower than that in Groups and ( $P<0.05$ ), while the proportions of feeding and drinking behavior frequencies in Group were significantly higher than those in Groups and ( $P<0.05$ ). 3) On days 1 and 7 of the experiment, the proportion of sitting resting behavior in Groups and was significantly lower than that in Group ( $P<0.05$ ). On day 7 of the experiment, the proportion of stretching resting behavior in Group was significantly lower than that in Group ( $P<0.05$ ). On day 14 of the experiment, the proportion of stretching resting behavior in Groups and was significantly lower than that in Group ( $P<0.05$ ). On days 1, 7, and 14 of the experiment, the proportion of prostrate resting behavior in Groups and was significantly higher than that in Group ( $P<0.05$ ). 4) The villus

height/crypt depth ratio in the jejunum of Group was significantly lower than that of Groups and ( $P<0.05$ ). It can be concluded that under an intermittently moderately hot environment of 31 °C, different RH levels had different effects on broiler growth performance, behavior, and jejunal morphology. High humidity had a greater impact on broiler ADG, ADFI, and jejunal morphology than low humidity, and the appropriate RH was 60%.

## Full Text

### Effects of Relative Humidity at Intermittent Moderate Temperature on Growth Performance, Behavior and Jejunum Morphological Structure of Broilers

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#### Abstract

This experiment was conducted to investigate the effects of relative humidity (RH) on growth performance, behavior and jejunum morphological structure of broilers at intermittent moderate temperature of 31 °C. A total of 180 Arbor Acres broilers at 22 days of age were randomly divided into three groups (Group I, II and III) and transferred to environmental control chambers, with six replicates per group and ten birds per replicate (half male and half female). The pre-test period lasted 7 days at 21 °C and 60% RH. During the 14-day formal test period starting at 29 days of age, the ambient temperature was maintained at 31 °C daily from 10:00 to 16:00 (6 hours), while RH in Groups I, II and III was set at 30%, 60% and 85%, respectively. The remaining time maintained the same temperature and humidity as the pre-test period. The results showed: 1) During days 1-14, average daily feed intake (ADFI), average daily gain (ADG) and average daily water intake in Group III were significantly lower than those in Group II ( $P<0.05$ ). 2) On days 1, 7 and 14 of the trial, the frequency proportion of resting behavior in Group II was significantly lower than in Groups I and III ( $P<0.05$ ), while the frequency proportions of feeding and drinking behaviors in Group II were significantly higher than in Groups I and III ( $P<0.05$ ). 3) On days 1 and 7, the proportion of sitting rest behavior in Groups I and III was significantly lower than in Group II ( $P<0.05$ ). On day 7, the proportion of stretching rest behavior in Group III was significantly lower than in Group II ( $P<0.05$ ), and on day 14, Groups I and III showed significantly lower proportions of stretching rest than Group II ( $P<0.05$ ). On days 1, 7 and 14, the proportion of prostration rest behavior in Groups I and III was significantly higher than in Group II ( $P<0.05$ ). 4) The villus height/crypt depth ratio of jejunum in Group III was significantly lower than in Groups I and II ( $P<0.05$ ). These findings indicate that at intermittent moderate temperature of 31 °C, different RH levels exert varying effects on broiler growth performance, behavior and jejunum

morphological structure, with high humidity having a greater impact on ADG, ADFI and jejunum morphology than low humidity, and the optimal RH being 60%.

**Keywords:** relative humidity; intermittent moderate environment; broilers; growth performance; behavior; jejunum morphological structure

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## Introduction

High temperature and humidity can damage gastrointestinal structure in poultry, cause lesions in liver and trachea, increase rectal and skin temperature, and lead to decreased production performance, seriously harming poultry growth and production [1-3]. In practice, with the widespread application of wet curtain ventilation technology in large-scale broiler houses in China [4], indoor temperatures rarely exceed 32 °C during summer. However, during early and late summer, intermittent environments exist in actual production where mornings and evenings are relatively cool while midday temperatures are moderately high. Current research has primarily focused on continuous high temperature heat stress environments at or above 32 °C, with insufficient attention paid to moderate heat environments (26-31 °C). Previous studies have found that when temperature exceeds the thermal comfort zone of poultry, individuals increase their distance from each other and exhibit increased panting or wing (leg) stretching frequencies [5]. Mack et al. [6] reported that under continuous heat stress (32.6 °C), laying hens showed decreased feed intake and body weight, along with increased wing-spreading, panting and drinking behaviors. Research has demonstrated that continuous heat stress (35 °C) damages duodenum and jejunum structure, reducing villus height, wet weight and dry weight [7-8]. At suitable temperatures, humidity may not significantly affect broiler growth and feed intake [9], and Milligan et al. [10] found that at 21 °C, RH between 48%-90% did not significantly affect broiler body weight, though results may differ under high temperature conditions. Studies have shown that under high temperature (>41 °C), poultry body temperature continuously increases with rising RH [11]. Yahav et al. [12] reported that 4-8 week-old broilers showed maximum feed intake and growth rate at 35 °C with 60%-65% RH. Our research group found that at 26 °C and 30 °C, the proportion of sitting rest time in broilers significantly decreased while prostration rest significantly increased [13], and continuous moderate heat stimulation differentially affected immune organ indices, small intestine morphological structure [14], and intestinal flora structure and diversity [15]. However, no studies have reported on the effects of RH on broiler behavior and jejunum morphological structure under intermittent moderate heat below 32 °C. Therefore, this experiment investigated the effects of different RH levels on broiler growth performance, behavior and jejunum morphological structure under intermittent moderate heat (31 °C) to explore broiler sensitivity to different humidity levels and provide scientific basis for humidity environmental control in production.

## 1.1 Experimental Animals and Design

A single-factor experimental design was adopted. A total of 180 healthy Arbor Acres (AA) broilers with similar body weight at 22 days of age were randomly divided into three groups (Groups I, II and III) and transferred to three environmental control chambers. Each group contained six replicates with ten birds per replicate (five males and five females). During a 7-day adaptation period, the temperature was maintained at 21 °C and RH at 60%. Starting at 29 days of age, Groups I, II and III were exposed to 31 °C with RH at 30%, 60% and 85%, respectively, daily from 10:00 to 16:00 (6 hours). For the remaining time, all three groups were maintained at 21 °C and 60% RH. The formal trial period lasted 14 days.

## 1.2 Management

Temperature and RH in the environmental control chambers were automatically controlled (precision  $\pm 1$  °C and  $\pm 7\%$ ) with no wind and 24-hour lighting. Broilers were raised in single-layer floor cages developed by our laboratory [16], with an infrared video camera (Best kebo BN-IPC4-3MD, Beijing) suspended above each cage to record broiler behavior. Videos were stored by a network video recorder (NVR). During the trial, broilers had free access to feed and water.

## 1.3 Diet Composition and Nutrient Levels

The experimental diet was a corn-soybean meal type diet formulated according to NRC (1994) standards and the *Chinese Feed Composition and Nutrient Value Table* (24th edition, 2013). The basal diet composition and nutrient levels are shown in Table 1 .

### 1.4.1 Growth Performance

During the formal trial period, daily feed intake and water consumption were recorded for each replicate. Body weight of broilers in each replicate was measured at the start, day 7 and day 14 to calculate average daily gain (ADG), average daily feed intake (ADFI), feed to gain ratio (F/G) and average daily water intake (ADWI).

### 1.4.2 Behavioral Measurement

Based on observation and referencing Hu Chunhong' s [17] behavioral classification method, broiler behaviors were categorized as described in Table 2 . Video collection was conducted for 6 hours on days 1, 7 and 14 of the trial, from which continuous 1-hour videos were selected for observation. Using instantaneous scan sampling, recordings were made every 30 seconds. Six birds per replicate (three males and three females) were observed, and the frequency of each behavior category for target birds was recorded. For data analysis, the proportion

of resting, feeding, drinking and preening behaviors to total behavior frequency was calculated, along with the percentage of each rest behavior to total rest behavior. All video observations and data recording were conducted by the same person, who observed one replicate from each group before moving to the next replicate.

### 1.4.3 Intestinal Morphological Measurement

On day 14 of the trial, six birds per group (three males and three females, one per replicate) were randomly selected. Approximately 2 cm of the mid-jejunum was collected, rinsed clean with physiological saline, and fixed in 4% paraformaldehyde solution for jejunum morphological structure measurement. Routine dehydration, paraffin embedding, sectioning, hematoxylin-eosin staining and slide mounting were performed. Six longest villi with intact crypt morphology were selected from each group to calculate average villus height, crypt depth and villus height/crypt depth (V/C) ratio.

## 1.5 Statistical Analysis

Behavioral data were initially processed using Excel with logarithmic transformation. Intestinal structure and growth performance data were first tested for normal distribution using the Univariate procedure, with data transformation applied for non-normal distributions. SAS 9.1 statistical software was then used for one-way ANOVA and Duncan's multiple comparison analysis. Results are expressed as "mean  $\pm$  standard deviation," with  $P < 0.05$  considered statistically significant.

## 2.1 Effects of RH on Growth Performance of Broilers at Intermittent Moderate Temperature

The effects of RH on growth performance are presented in Table 3 . During days 1-7, ADFI and ADG in Group III were significantly lower than in Group II ( $P < 0.05$ ), while ADWI in Groups I and III was significantly lower than in Group II ( $P < 0.05$ ). During days 8-14, ADFI in Group III was significantly lower than in Group II ( $P < 0.05$ ), and ADWI in Group I was significantly lower than in Group II ( $P < 0.05$ ). Over days 1-14, ADFI, ADG and ADWI in Group III were significantly lower than in Group II ( $P < 0.05$ ). RH showed no significant effect on F/G during the trial period ( $P > 0.05$ ).

## 2.2 Effects of RH on Behavior of Broilers at Intermittent Moderate Temperature

The effects of RH on behavior are presented in Table 4 and Table 5 . As shown in Table 4, on days 1, 7 and 14, the frequency proportion of resting behavior in Group II was significantly lower than in Groups I and III ( $P < 0.05$ ), with no significant difference between Groups I and III ( $P > 0.05$ ). The frequency

proportion of feeding behavior in Group II was significantly higher than in Groups I and III on days 1, 7 and 14 ( $P < 0.05$ ), with proportions decreasing over time in all groups. Drinking behavior frequency proportion in Group II was significantly higher than in Groups I and III on days 1, 7 and 14 ( $P < 0.05$ ), with no significant difference between Groups I and III ( $P > 0.05$ ). On day 1, preening behavior frequency proportion in Group II was significantly higher than in Groups I and III ( $P < 0.05$ ).

As shown in Table 5, the proportion of sitting rest behavior gradually decreased with trial days, while stretching rest and prostration rest proportions gradually increased, with prostration rest accounting for the largest proportion. On days 1 and 7, the proportion of sitting rest behavior in Groups I and III was significantly lower than in Group II ( $P < 0.05$ ). On day 7, the proportion of stretching rest behavior in Group III was significantly lower than in Group II ( $P < 0.05$ ), and on day 14, Groups I and III showed significantly lower proportions of stretching rest than Group II ( $P < 0.05$ ). On days 1, 7 and 14, the proportion of prostration rest behavior in Groups I and III was significantly higher than in Group II ( $P < 0.05$ ).

### **2.3 Effects of RH on Jejunum Morphological Structure of Broilers at Intermittent Moderate Temperature**

The effects of RH on jejunum morphological structure are presented in Table 6. Villus height in Group II was greater than in Groups I and III, with Group III showing the lowest value, but differences among groups were not significant ( $P > 0.05$ ). Crypt depth in Group III was greater than in Groups I and II, with Group II showing the lowest value, but differences were not significant ( $P > 0.05$ ). The V/C ratio of jejunum in Group III was significantly lower than in Groups I and II ( $P < 0.05$ ), with no significant difference between Groups I and II ( $P > 0.05$ ).

### **3.1 Effects of RH on Growth Performance of Broilers at Intermittent Moderate Temperature**

Animal growth performance reflects health status and environmental comfort, serving as one of the animal welfare standards. Studies have found that constant high temperature (35 °C) decreases poultry production performance [18], and Zhou et al. [19] reported that high humidity (85%) under intermittent 31 °C significantly reduced broiler ADFI and ADG. Yahav [20] reported that at 28 °C and 30 °C, broiler weight gain reached maximum at 60%-65% RH. This experiment found that high humidity group showed significantly lower ADFI and ADG than moderate humidity group. During days 1-7, both low and high humidity groups showed significantly lower ADWI than moderate humidity group; during days 7-14, low humidity group showed significantly lower ADWI; and during days 1-14, high humidity group showed significantly lower ADWI. These results indicate that at intermittent moderate temperature of 31 °C, feed intake and water consumption reached maximum at 60% RH. The possible reason is that under

high temperature, high humidity hinders evaporative heat dissipation in broilers [20-21], causing body temperature imbalance and metabolic disorders, leading to significantly reduced ADFI, ADG and ADWI. While low humidity promotes evaporative heat dissipation, excessively low RH may cause dehydration [22], harming broiler health and growth, and limiting water balance regulation [20]. In this study, low humidity group showed lower ADWI than moderate humidity group, possibly because high temperature with low humidity caused excessively fast evaporative heat dissipation, making broilers uncomfortable and disrupting water balance regulation, thus reducing drinking behavior and ADWI.

### 3.2 Effects of RH on Behavior of Broilers at Intermittent Moderate Temperature

Research indicates that under heat stress, poultry reduce feed intake to decrease metabolic heat production and heat dissipation [23-24]. This experiment found that moderate humidity group showed significantly higher feeding behavior frequency proportion than low and high humidity groups, suggesting that both low and high humidity exacerbate heat stress degree under intermittent 31 °C. Either decreased or increased humidity may inhibit heat dissipation, leading to reduced feed intake. The moderate humidity group showed significantly higher proportions of resting and drinking behavior frequencies than low and high humidity groups, possibly because moderate humidity caused less heat stress, resulting in greater activity, more feeding and more drinking. On day 1, moderate humidity group showed significantly higher preening behavior frequency proportion than the other two groups, consistent with our research group member Hu Chunhong's [17] findings, indicating that broilers exhibit more preening behavior under comfortable conditions.

Under high temperature conditions, animals first dissipate heat through physical regulation including physiological and behavioral mechanisms, such as increasing skin blood flow or showing body stretching and panting [25]. Pickel et al. [26] found that broilers primarily exhibit sitting rest behavior under thermal comfort. In this experiment, on days 1 and 7, low and high humidity groups showed significantly lower proportions of sitting rest behavior than moderate humidity group, while on days 1, 7 and 14, they showed significantly higher proportions of prostration rest than moderate humidity group. These results indicate that compared with moderate humidity, both low and high humidity significantly reduced broiler comfort under intermittent 31 °C. Richards [27] found that panting increases evaporative heat dissipation. As poultry house temperature increases, respiratory frequency increases with higher humidity [28]. Gerken et al. [29] found that under heat stress, broilers increase contact area between the bare area under wings and air, conducting heat through blood vessels in the bare skin area to dissipate heat and reduce body temperature. This experiment found that on day 7, high humidity group showed significantly lower proportion of stretching rest behavior than moderate humidity group, and on day 14, both low and high humidity groups were significantly lower than moderate humidity group.

These findings suggest that compared with moderate humidity, high humidity had greater impact on stretching rest behavior proportion. Under moderate humidity, broilers could maintain body temperature balance by stretching wings to expose bare areas for heat dissipation, while under high humidity they primarily relied on prostration and accelerated respiratory evaporation for heat dissipation.

### 3.3 Effects of RH on Jejunum Morphological Structure of Broilers at Intermittent Moderate Temperature

The small intestine is the primary site for nutrient absorption and transport in the body, with the jejunum being the longest segment where normal structure forms the basis for nutrient digestion and absorption [30]. Important indicators for measuring jejunum digestion and absorption function include villus height, crypt depth and V/C ratio [31]. Studies have shown that villus height directly affects animal growth and development, as shortened villi reduce contact area with intestinal chyme, leading to decreased absorption function [32]. Crypts are tubular glands formed by epithelial invagination at villus roots, with crypt depth reflecting epithelial cell generation rate; shallower crypts indicate increased mature epithelial cell numbers and enhanced secretory function [33]. The V/C ratio comprehensively reflects small intestine digestion and absorption capacity [34]. In this experiment, compared with moderate humidity, high humidity significantly reduced jejunum V/C ratio, while low humidity showed no significant difference from moderate humidity, indicating that high humidity had greater impact on jejunum and significantly reduced its digestion and absorption capacity. The decreased V/C ratio may result from mucosal damage leading to weakened digestion and absorption function.

In summary, compared with moderate humidity, both low and high humidity increased prostration rest behavior and decreased feeding, drinking behavior and ADWI in broilers. Additionally, high humidity reduced jejunum digestion and absorption capacity, leading to decreased ADG and ADFI. Therefore, under intermittent moderate heat environment, RH affects broiler growth performance, sitting rest behavior, prostration rest behavior, stretching rest behavior, feeding behavior, drinking behavior and jejunum morphological structure. High humidity has greater impact on broiler ADG, ADFI and jejunum morphology than low humidity, making 60% RH the optimal level.

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