

## Effects of Cyclic Heat Stress on Respiratory Function, Growth Performance, and Liver Antioxidant Function in Yellow-Feathered Broilers: Postprint

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### Abstract

This study was conducted to investigate the effects of cyclic heat stress on respiratory function, growth performance, and liver antioxidant function in yellow-feathered broilers. A total of 144 male yellow-feathered broilers at 35 days of age with similar body weight were randomly divided into two groups: a normal temperature group and a cyclic heat stress group, with 12 replicates per group and 6 birds per replicate. Both groups were fed a basal diet. The normal temperature group was maintained at  $(26.0 \pm 0.5)^\circ\text{C}$ , while the cyclic heat stress group was subjected to the following temperature regimen:  $26^\circ\text{C}$  from 20:00 to 08:00 the following day,  $30^\circ\text{C}$  from 08:00 to 12:00 and from 16:00 to 20:00, and  $34^\circ\text{C}$  from 12:00 to 16:00. The experimental period lasted 6 days. The results showed that after 2 days of cyclic heat stress, the cyclic heat stress group exhibited significantly higher comb temperature, under-wing temperature, rectal temperature, respiratory rate, feed-to-gain ratio (F/G), and liver protein carbonyl content compared with the normal temperature group ( $P < 0.05$ ), while average daily feed intake (ADFI), average daily gain (ADG), and liver catalase (CAT) and superoxide dismutase (SOD) activities were significantly lower ( $P < 0.05$ ). After 6 days of cyclic heat stress, the cyclic heat stress group had significantly higher comb temperature, respiratory rate, F/G, and liver protein carbonyl content ( $P < 0.05$ ), whereas ADFI, ADG, liver reduced glutathione content, and CAT, SOD, and glutathione peroxidase activities were significantly lower than those in the normal temperature group ( $P < 0.05$ ). In conclusion, cyclic heat stress can increase body temperature, accelerate respiratory rate, and reduce growth performance and liver antioxidant function in yellow-feathered broilers.

## Full Text

# Effects of Cyclic Heat Stress on Breathing Function, Growth Performance and Liver Antioxidant Function of Yellow-Feathered Broilers

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## Abstract

This study was designed to investigate the effects of cyclic heat stress on breathing function, growth performance, and liver antioxidant function in yellow-feathered broilers. A total of 144 thirty-five-day-old male yellow-feathered broilers with similar body weight were randomly divided into two groups: a normal temperature group and a cyclic heat stress group. Each group comprised 12 replicates with 6 birds per replicate. Both groups were fed a basal diet. The normal temperature group was maintained at (26.0±0.5) °C, while the cyclic heat stress group was exposed to a temperature cycle of 26 °C from 20:00 to 08:00, 30 °C from 08:00 to 12:00 and 16:00 to 20:00, and 34 °C from 12:00 to 16:00. The experimental period lasted 6 days. The results showed that after 2 days of cyclic heat stress, the cyclic heat stress group exhibited significantly higher comb temperature, wing temperature, rectal temperature, breathing rate, feed-to-gain ratio (F/G), and liver protein carbonyl content compared to the normal temperature group (P<0.05). Conversely, average daily feed intake (ADFI), average daily gain (ADG), and liver catalase (CAT) and superoxide dismutase (SOD) activities were significantly lower (P<0.05). After 6 days of cyclic heat stress, the cyclic heat stress group showed significantly higher comb temperature, breathing rate, F/G, and liver protein carbonyl content (P<0.05), while ADFI, ADG, and liver reduced glutathione content and CAT, SOD, and glutathione peroxidase activities were significantly lower (P<0.05). In conclusion, cyclic heat stress elevated body temperature, accelerated breathing rate, and impaired growth performance and liver antioxidant function in yellow-feathered broilers.

**Keywords:** yellow-feathered broiler; cyclic heat stress; breathing function; growth performance; antioxidant function

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## Introduction

Heat stress represents a non-specific physiological defense response of animals to hot environmental conditions. Due to their feather coverage and lack of sweat glands, poultry have difficulty dissipating heat, making them susceptible to heat

stress, which can induce oxidative stress and adversely affect the liver. Previous studies have demonstrated that heat stress inhibits animal growth, with this inhibitory effect being associated with oxidative damage. Research by Fu Xubin showed that heat stress reduces liver antioxidant function, causing pathological damage to hepatocytes, including granular and fatty degeneration. In 2016, China's yellow-feathered broiler production exceeded 4 billion birds, accounting for approximately half of total broiler production. Current production systems predominantly involve free-range farming by smallholders with poor environmental control, making heat stress a common problem during summer. However, domestic research on heat stress in yellow-feathered broilers remains limited, with most studies employing constant high-temperature protocols that differ substantially from actual daily temperature fluctuations in production settings. This experiment simulated summer environmental temperatures in East China to establish a heat stress model and investigate the effects of cyclic heat stress on breathing function, growth performance, and liver antioxidant function in yellow-feathered broilers, providing a basis for understanding oxidative stress-induced damage mechanisms and their extension to mitochondrial injury pathways.

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## 1. Materials and Methods

### 1.1 Experimental Design

A total of 144 healthy thirty-five-day-old male yellow-feathered broilers with a body weight of  $(1,360 \pm 10)$  g were randomly allocated to two groups: a normal temperature group and a cyclic heat stress group. Each group contained 12 replicates with 6 birds per replicate. Both groups received a basal diet. The normal temperature group was maintained at  $(26.0 \pm 0.5)$  °C, while the cyclic heat stress group was exposed to a temperature cycle simulating summer conditions in East China: 26 °C from 20:00 to 08:00, 30 °C from 08:00 to 12:00 and 16:00 to 20:00, and 34 °C from 12:00 to 16:00. This temperature pattern was provided by Jiangsu Lihua Animal Husbandry Co., Ltd. The experimental period lasted 6 days.

### 1.2 Feeding Management

The experiment was conducted in environmental control chambers at the Yizheng Base of the Poultry Institute, Chinese Academy of Agricultural Sciences. Birds were housed in three-tier cages (100 cm × 80 cm × 45 cm) with ad libitum access to feed and water. Relative humidity was maintained at  $(60 \pm 5)\%$ . The temperature cycle for the heat stress group is illustrated in [Figure 1: see original paper] (temperature transitions were completed within 5 minutes). Lighting was provided 24 hours daily. Birds were weighed at the start of the experiment, and all groups were fed a basal diet formulated according to the Chinese Feeding Standard for Chickens (NY/T 33-2004). The composition

and nutrient levels of the basal diet are presented in . Environmental control chambers were manufactured by Beijing Kulan Technology Co., Ltd., and yellow-feathered broilers were purchased from Yangzhou Lihua Livestock Co., Ltd.

### 1.3 Measurement Indicators

**1.3.1 Breathing Function** After 2 and 6 days of cyclic heat stress, comb temperature and wing temperature were measured using a non-contact infrared thermometer (Model AR550, Xima Instrument Co., Ltd.). Rectal temperature was measured using an animal rectal thermometer (Model HRQ-S2009, Zhengzhou Haorunqi Electronic Technology Co., Ltd.). Each parameter was measured three times and averaged. Breathing rate was determined by filming each replicate with a mini camera (Model SQ8, Shenzhen Kainikang Technology Co., Ltd.) between 12:30 and 13:30. The number of breaths per minute was counted for three birds per replicate during a period near 13:00, with continuous observation for 5 minutes, and the average value was calculated.

**1.3.2 Growth Performance** Feed weight was recorded at each feeding. Body weight and residual feed weight were recorded for each replicate at the start and end of the experiment to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G) for days 1-2, days 3-6, and days 1-6.

**1.3.3 Liver Antioxidant Function** After 2 and 6 days of cyclic heat stress, one bird was randomly selected from each replicate, and liver tissue was collected. Approximately 0.1 g of liver tissue was weighed, homogenized, and diluted according to kit instructions. The homogenate was analyzed for catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activities, as well as reduced glutathione (GSH), malondialdehyde (MDA), and protein carbonyl (PC) contents. All assay kits were provided by Nanjing Jiancheng Bioengineering Institute.

### 1.4 Data Processing

Data were initially processed using Excel 2013 and then subjected to one-way ANOVA using the Univariate procedure in SPSS 20.0 software. Duncan's multiple comparison test was used for mean separation. Results are expressed as means  $\pm$  standard deviation, with  $P < 0.05$  considered statistically significant.

## 2. Results

### 2.1 Effects of Cyclic Heat Stress on Breathing Function in Yellow-Feathered Broilers

As shown in , after 2 days of cyclic heat stress, comb temperature, wing temperature, rectal temperature, and breathing rate in the cyclic heat stress group were significantly higher than those in the normal temperature group ( $P < 0.05$ ). After 6 days, comb temperature and breathing rate remained significantly higher in the cyclic heat stress group ( $P < 0.05$ ).

### 2.2 Effects of Cyclic Heat Stress on Growth Performance in Yellow-Feathered Broilers

As shown in , the cyclic heat stress group exhibited significantly lower ADFI and ADG during days 1-2, lower ADG during days 3-6, and lower ADFI and ADG during days 1-6 compared to the normal temperature group ( $P < 0.05$ ). The F/G during days 1-2 and days 1-6 was significantly higher in the cyclic heat stress group ( $P < 0.05$ ).

### 2.3 Effects of Cyclic Heat Stress on Liver Antioxidant Function in Yellow-Feathered Broilers

As shown in , after 2 days of cyclic heat stress, liver MDA content and CAT and SOD activities were significantly lower in the cyclic heat stress group ( $P < 0.05$ ), while protein carbonyl content was significantly higher ( $P < 0.05$ ). After 6 days, liver MDA and GSH contents and CAT, SOD, and GSH-Px activities were significantly lower in the cyclic heat stress group ( $P < 0.05$ ), while protein carbonyl content was significantly higher ( $P < 0.05$ ).

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## 3. Discussion

### 3.1 Effects of Cyclic Heat Stress on Breathing Function in Yellow-Feathered Broilers

High-temperature environments trigger physiological regulatory responses, such as accelerated breathing and increased peripheral blood flow, to maintain body temperature. Body surface temperature represents an adaptive physiological response to environmental conditions and serves as a marker for evaluating heat stress. Bohmanova et al. reported that heat stress in dairy cows significantly increased rectal temperature, body surface temperature, and breathing rate. Similarly, Li et al. demonstrated that heat-stressed broilers increased breathing rate and pulmonary ventilation to enhance evaporative and convective heat loss through the respiratory tract. Our results showed that after 2 days of cyclic heat stress, comb temperature, wing temperature, rectal temperature, and breathing rate were significantly higher in the cyclic heat stress group. After 6 days, comb

temperature and breathing rate remained significantly elevated, indicating that cyclic heat stress increased breathing rate and body surface temperature in yellow-feathered broilers, consistent with previous findings. Notably, after 6 days, wing temperature and rectal temperature showed no significant differences between groups, suggesting that yellow-feathered broilers may have begun to adapt to cyclic heat stress, exhibiting improved thermoregulatory capacity.

### **3.2 Effects of Cyclic Heat Stress on Growth Performance in Yellow-Feathered Broilers**

Heat stress is a primary factor affecting poultry growth performance, altering physiological functions and causing metabolic disorders that manifest as reduced weight gain, slower growth, and decreased resistance. Sohail et al. reported that chronic heat stress significantly reduced body weight by 32.6% and increased F/G by 25.6% in 42-day-old broilers. Deng et al. and Mashaly et al. suggested that reduced feed intake is likely the root cause of impaired growth performance, leading to decreased body weight, feed efficiency, egg production, and egg quality. Our results showed that during days 3–6, ADFI decreased by 19.32% and F/G increased by 10.94% in the cyclic heat stress group compared to the normal temperature group. ADFI and ADG were significantly reduced, and F/G was significantly increased across all stages, demonstrating that cyclic heat stress reduced weight gain, increased feed conversion ratio, and impaired growth performance in yellow-feathered broilers, consistent with previous research.

### **3.3 Effects of Cyclic Heat Stress on Liver Antioxidant Function in Yellow-Feathered Broilers**

Moderate levels of free radicals are harmless or even beneficial, but excessive free radicals produced under stress conditions disrupt normal physiological processes and metabolism. Free radical scavenging primarily depends on antioxidant enzymes, including SOD, CAT, and GSH-Px. MDA is the end product of lipid peroxidation, while protein carbonyl represents the end product of protein oxidation. Studies have shown that heat stress significantly reduces GSH-Px activity in the liver, leading to increased MDA content. Li et al. reported that heat stress significantly increased liver peroxidation in broilers, while Chen et al. found that oxidative stress increased MDA content and decreased SOD activity after 48 hours. Our results demonstrated that after 2 days of cyclic heat stress, liver CAT and SOD activities were significantly reduced, while protein carbonyl content was significantly increased. After 6 days, liver CAT, SOD, and GSH-Px activities and GSH content were significantly reduced, while protein carbonyl content remained significantly elevated. These findings indicate that cyclic heat stress impaired antioxidant enzyme activity and reduced antioxidant function in yellow-feathered broilers, consistent with previous studies. Notably, antioxidant function did not improve after 6 days of cyclic heat stress; instead, GSH-Px activity decreased further, suggesting that cyclic heat stress continued to cause damage and that yellow-feathered broilers had not yet adapted to

the thermal environment. Interestingly, liver MDA content was lower than the normal temperature group at both stages, which differs from previous research findings.

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#### 4. Conclusion

Cyclic heat stress elevated body temperature, accelerated breathing rate, and impaired growth performance and liver antioxidant function in yellow-feathered broilers.

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