

## Effects of Chronic Heat Stress on Growth Performance, Organ Indices, Serum Biochemical Indices, and Antioxidant Function in Yellow-Feathered Broilers (Postprint)

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### Abstract

This study aimed to investigate the effects of continuous heat stress on growth performance, organ indices, serum biochemical indices, and antioxidant function in yellow-feathered broilers. A total of 192 healthy 35-day-old yellow-feathered broilers were randomly allocated to a thermoneutral group and a continuous heat stress group, with 8 replicates per group and 12 birds per replicate. The thermoneutral group was maintained at  $(26\pm 0.5)$  °C, while the continuous heat stress group was subjected to constant 34 °C; relative humidity was approximately 55% in both groups. The experimental period lasted 14 days. The results showed that after 3 days of heat stress, serum uric acid (UA), triglyceride (TG), and calcium ion ( $\text{Ca}^{2+}$ ) concentrations, as well as hepatic glutathione peroxidase (GSH-Px) activity and nitric oxide (NO) content in the continuous heat stress group were significantly lower than those in the thermoneutral group ( $P < 0.05$ ). After 7 days of heat stress, serum total cholesterol (T-CHO) and high-density lipoprotein (HDL) concentrations and hepatic NO content in the continuous heat stress group were significantly higher than those in the thermoneutral group ( $P < 0.05$ ), whereas serum TG and NO concentrations and catalase (CAT) and superoxide dismutase (SOD) activities were significantly lower ( $P < 0.05$ ). After 14 days of heat stress, body weight (BW), average daily gain (ADG), and average daily feed intake (ADFI) in the continuous heat stress group were significantly lower than those in the thermoneutral group, while feed-to-gain ratio (F/G) was significantly higher; serum glucose (GLU) and TG concentrations were also significantly higher ( $P < 0.05$ ). In conclusion, continuous heat stress reduces growth performance, affects lipid metabolism, and decreases antioxidant function in yellow-feathered broilers.

## Full Text

### Abstract

This experiment was conducted to investigate the effects of persistent heat stress on growth performance, organ indices, serum biochemical indices, and antioxidant function in yellow-feathered broilers. A total of 192 healthy 35-day-old yellow-feathered broilers were randomly divided into two groups: a normal temperature group and a persistent heat stress group, with 8 replicates per group and 12 birds per replicate. The normal temperature group was maintained at  $(26\pm 0.5)$  °C, while the persistent heat stress group was subjected to continuous heat stress at 34 °C. Relative humidity was maintained at approximately 55% in both groups, and the experimental period lasted 14 days. The results showed that on day 3 of heat stress, serum uric acid (UA), triglyceride (TG), and calcium ion ( $\text{Ca}^{2+}$ ) concentrations, as well as hepatic glutathione peroxidase (GSH-Px) activity and nitric oxide (NO) content, were significantly lower in the heat stress group compared to the normal group ( $P < 0.05$ ). On day 7 of heat stress, serum total cholesterol (T-CHO) and high-density lipoprotein (HDL) concentrations and hepatic NO content were significantly higher in the heat stress group ( $P < 0.05$ ), while serum TG and NO concentrations and catalase (CAT) and superoxide dismutase (SOD) activities were significantly lower ( $P < 0.05$ ). On day 14 of heat stress, body weight (BW), average daily gain (ADG), and average daily feed intake (ADFI) were significantly lower in the heat stress group, while feed-to-gain ratio (F/G) was significantly higher ( $P < 0.05$ ). Serum glucose (GLU) and TG concentrations were also significantly higher in the heat stress group on day 14 ( $P < 0.05$ ). In summary, persistent heat stress reduces growth performance, affects lipid metabolism, and impairs antioxidant function in yellow-feathered broilers.

**Keywords:** yellow-feathered broilers; persistent heat stress; growth performance; organ indices; serum biochemical indices; antioxidant function

### Introduction

High ambient temperature is a critical factor affecting animal production and reproduction, causing cellular and tissue damage, impeding organ development, and reducing antioxidant capacity. Blood parameters change in response to tissue cell function and metabolic alterations, making serum biochemical indices commonly used to reflect nutritional and metabolic status. Numerous studies have reported the effects of heat stress on physiological and biochemical parameters such as blood glucose, lipids, and apolipoproteins. Therefore, examining heat stress impacts on blood indices provides a foundation for exploring the mechanisms of heat stress-induced damage.

Extensive research on heat stress has been conducted both domestically and internationally. Sun et al. demonstrated that heat stress alters blood parameters and affects lipid metabolism. Liu et al. showed that acute heat stress

increases serum triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) concentrations in broilers, activating fatty acid synthesis-related enzymes and promoting fat deposition. Huang reported that heat stress reduces hepatic catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activities, disrupting redox balance. Jin et al. and Zhang et al. found that heat stress impairs antioxidant function, though animals gradually establish a new oxidative balance as heat stress duration extends.

In China, yellow-feathered broilers are primarily raised in free-range systems by small farmers with poor environmental control, making them highly susceptible to heat stress during summer. However, most current research focuses on white-feathered broilers such as Ross 308, with limited studies on yellow-feathered broilers. Therefore, this experiment aimed to investigate the effects of persistent heat stress on growth performance, organ indices, serum biochemical indices, and antioxidant function in yellow-feathered broilers, providing insights for mitigating heat stress damage in large-scale yellow-feathered broiler production.

## Materials and Methods

### Experimental Design

A total of 192 healthy 35-day-old medium-growing “Youma” yellow-feathered broilers with similar body weight were randomly allocated to two groups: a normal temperature group and a persistent heat stress group. Each group consisted of 8 replicates with 12 birds per replicate. Both groups were fed a basal diet. The normal temperature group was maintained at 26 °C, while the persistent heat stress group was subjected to continuous heat stress at 34 °C. Relative humidity was approximately 55% in both groups. The experimental period lasted 14 days, with samples collected on days 3, 7, and 14 of heat stress.

### Animal Management

The experiment was conducted at the experimental base of the Poultry Institute, Chinese Academy of Agricultural Sciences. Birds were housed in three-tier cages (120 cm × 80 cm × 60 cm length × width × height) and managed according to yellow-feathered broiler feeding standards. The environmental control system was provided by Beijing Kulan Technology Co., Ltd. Yellow-feathered broilers were purchased from Yangzhou Lihua Livestock and Poultry Co., Ltd. The basal diet was formulated according to the Chinese “Feeding Standard of Chicken” (NY/T 33-2004). The composition and nutrient levels of the basal diet are presented in .

## Sample Collection and Analysis

**Growth Performance** Initial body weight was recorded for each replicate. Feed weight was measured at each feeding, and body weight and remaining feed weight were recorded before sample collection to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) during days 1-14 of heat stress.

**Organ Indices** On days 3, 7, and 14 of heat stress, two birds were randomly selected from each replicate, weighed, and slaughtered. The abdominal skin was opened, and abdominal fat pad width was measured at three locations (both ends and middle) using vernier calipers and averaged. The liver, spleen, pectoral muscle, and gastrocnemius muscle were dissected and weighed to calculate organ indices.

**Serum Biochemical Indices** On days 3, 7, and 14 of heat stress, two birds per replicate were randomly selected for blood collection from the wing vein into procoagulant tubes. After clotting and centrifugation, serum was separated and stored at -20 °C. Serum concentrations of glucose (GLU), uric acid (UA), urea nitrogen (UN), TG, total cholesterol (T-CHO), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and calcium ions ( $\text{Ca}^{2+}$ ) were determined using a UniCel DxC 800 Synchron automatic biochemical analysis system (Beckman Coulter, USA). Reagent kits were purchased from Zhongshan Biaoja Biotechnology Co., Ltd.

**Serum Antioxidant Enzyme Activities** Blood samples collected on days 3, 7, and 14 of heat stress were processed as described above. Serum CAT, SOD, and GSH-Px activities and nitric oxide (NO) content were measured using reagent kits from Nanjing Jiancheng Bioengineering Institute.

**Hepatic Antioxidant Enzyme Activities** On days 3, 7, and 14 of heat stress, two birds per replicate were randomly selected. Small liver samples were collected from the same anatomical location, placed in cryovials, and subjected to homogenization and dilution for measurement of hepatic CAT, SOD, and GSH-Px activities and NO content using reagent kits from Nanjing Jiancheng Bioengineering Institute.

## Statistical Analysis

Data were initially processed using Excel 2013 and then analyzed using t-tests in SPSS 20.0 software. Results are expressed as mean  $\pm$  standard deviation. Differences were considered significant at  $P < 0.05$  and marginally significant at  $0.05 < P < 0.10$ .

## Results

### Effects of Persistent Heat Stress on Growth Performance

As shown in , during days 1-14 of heat stress, body weight, ADG, and ADFI were significantly lower in the persistent heat stress group compared to the normal group ( $P<0.05$ ), while F/G was significantly higher ( $P<0.05$ ).

### Effects of Persistent Heat Stress on Organ Indices

As shown in , on day 3 of heat stress, spleen index was significantly lower in the persistent heat stress group compared to the normal group ( $P<0.05$ ). No significant differences were observed for other organs or time points.

### Effects of Persistent Heat Stress on Serum Biochemical Indices

As shown in , on day 3 of heat stress, serum UA, TG, and  $Ca^{2+}$  concentrations were significantly lower in the persistent heat stress group ( $P<0.05$ ). On day 7, serum T-CHO and HDL concentrations were significantly higher ( $P<0.05$ ), while serum TG concentration was significantly lower ( $P<0.05$ ). On day 14, serum GLU and TG concentrations were significantly higher in the persistent heat stress group ( $P<0.05$ ), while serum T-CHO concentration showed a tendency to be higher ( $P=0.098$ ).

### Effects of Persistent Heat Stress on Serum Antioxidant Function

As shown in , on day 3 of heat stress, serum CAT activity showed a tendency to be lower in the persistent heat stress group ( $P=0.065$ ). On day 7, serum CAT and SOD activities and NO content were significantly lower ( $P<0.05$ ).

### Effects of Persistent Heat Stress on Hepatic Antioxidant Function

As shown in , on day 3 of heat stress, hepatic GSH-Px activity and NO content were significantly lower in the persistent heat stress group ( $P<0.05$ ), while hepatic SOD activity showed a tendency to be lower ( $P=0.060$ ). On day 7, hepatic NO content was significantly higher ( $P<0.05$ ), while hepatic CAT activity showed a tendency to be higher ( $P=0.072$ ).

## Discussion

### Effects of Persistent Heat Stress on Growth Performance

Adequate feed intake is essential for animal survival and optimal production potential. Heat stress reduces feed intake, leading to altered physiological function, metabolic disorders, decreased body weight, compromised immune function, and even mortality as stress intensity increases. Deng et al. and Lin et al. demonstrated that reduced feed intake is the primary cause of impaired

growth performance under heat stress, directly decreasing body weight, feed utilization, egg production, and egg quality. Sohail et al. also reported that heat stress significantly reduces body weight and feed efficiency in 42-day-old broilers, adversely affecting production. Our results show that after 14 days of heat stress, ADG and ADFI were significantly reduced while F/G was significantly increased, indicating that persistent heat stress impaired feed intake, growth, and feed utilization, thereby reducing growth performance. These findings are consistent with previous studies.

### **Effects of Persistent Heat Stress on Organ Indices**

Organ indices are important indicators of systemic development and directly affect metabolic levels. The spleen is a crucial immune organ in poultry. Guo et al. found that prolonged heat stress impedes organ development, significantly reducing immune organ weight, particularly spleen weight. Liu et al. also demonstrated that heat stress reduces spleen weight in broilers. However, Wang et al. reported no significant effects of heat stress on organ development in mice, suggesting that discrepancies may be related to experimental animals and conditions. Our results show that spleen index was significantly lower in the heat stress group on day 3, but no significant differences were observed for other organs or time points. This suggests that under our experimental conditions, persistent heat stress had no significant effect on organ development, which differs from previous findings. This discrepancy may be attributed to the use of yellow-feathered broilers, which may have greater resistance and adaptability to adverse environmental conditions.

### **Effects of Persistent Heat Stress on Serum Biochemical Indices**

Serum biochemical indices are commonly used to reflect metabolic status and organ function changes in animals and are widely applied in heat stress research. Serum GLU, UA, TG, and T-CHO concentrations reflect carbohydrate, protein, and lipid metabolism, while serum UN concentration indicates amino acid balance and protein metabolism. Serum  $\text{Ca}^{2+}$  concentration reflects blood pH changes; when pH decreases, bound calcium dissociates, increasing  $\text{Ca}^{2+}$  levels. Guo reported that heat stress elevates serum GLU concentration, increases protein degradation, and enhances fat synthesis as the primary energy storage method. Wang et al. demonstrated that heat stress increases serum GLU concentration, likely due to glucocorticoid-mediated acceleration of protein catabolism and gluconeogenesis. Our results show that on day 3 of heat stress, serum TG and  $\text{Ca}^{2+}$  concentrations decreased; on day 7, serum TG decreased while HDL and T-CHO increased; and on day 14, serum TG significantly increased. These findings indicate that persistent heat stress affected lipid metabolism, but the opposing results for serum TG at days 3, 7, and 14 prevent definitive conclusions about the specific effects on lipid metabolism, warranting further investigation. The decreased serum  $\text{Ca}^{2+}$  concentration on day 3 may result from accelerated respiration due to persistent heat stress, increasing  $\text{CO}_2$  excretion and shifting

the carbonic acid-bicarbonate ( $\text{H}_2\text{CO}_3 - \text{HCO}_3^-$ ) equilibrium, thereby increasing blood pH. This is consistent with previous findings.

### **Effects of Persistent Heat Stress on Serum Antioxidant Function**

Under heat stress conditions, excessive free radicals are produced, disrupting redox balance and causing metabolic disorders. Free radical scavenging primarily depends on antioxidant enzymes including SOD, CAT, and GSH-Px. Li et al. reported that heat stress significantly increased lipid peroxidation, elevated serum malondialdehyde (MDA) concentration, and reduced serum GSH-Px activity, impairing antioxidant function. NO is an important regulator of cardiovascular function and a neurotransmitter with diverse physiological roles. During heat stress, NO causes vasodilation, mediates cardiopulmonary function, and regulates body temperature. Wang et al. demonstrated that heat stress significantly reduced serum GSH-Px activity, leading to increased serum MDA concentration. MDA concentration increased and SOD activity decreased after 48 hours of stress. Our results show that on day 3, serum CAT activity tended to be lower in the heat stress group, while on day 7, serum CAT and SOD activities were significantly lower, indicating that persistent heat stress reduced serum antioxidant function. The progressive decline in serum antioxidant enzyme activities with prolonged heat stress suggests that persistent heat stress exacerbates antioxidant impairment over time, consistent with previous studies. The lack of significant differences in serum antioxidant enzyme activities on day 14 may indicate that birds gradually adapted to the thermal environment and established a new redox balance, allowing antioxidant function to recover. Interestingly, serum NO concentration was significantly lower in the heat stress group on day 7, which differs from previous findings and requires further investigation.

### **Effects of Persistent Heat Stress on Hepatic Antioxidant Function**

Our results demonstrate that on day 3, hepatic GSH-Px activity was significantly lower in the heat stress group; on day 7, hepatic NO content was significantly higher; and on day 14, no significant differences in hepatic antioxidant enzyme activities were observed. These findings indicate that persistent heat stress reduced hepatic antioxidant enzyme activity and increased NO content, but as duration extended, birds may have gradually adapted and established a new redox balance, eliminating differences in enzyme activities. These results are consistent with Wang et al. and Wang et al. However, the significantly lower hepatic NO content on day 3 in the heat stress group differs from previous studies but aligns with our serum NO results. We believe this phenomenon is not due to measurement error but may be related to experimental animals, age, and other conditions, requiring further verification.

## Conclusion

Persistent heat stress reduces growth performance, affects lipid metabolism, and impairs antioxidant function in yellow-feathered broilers.

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