Effects of Relative Humidity and Intermittent Heat Stress on Immune Function in Broiler Chickens: Postprint

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Date: 2017-11-07T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of relative humidity (RH) and intermittent partial heat treatment on the immune function of broiler chickens. A 2$×$3 factorial design was employed with two partial heat levels (26 and 31 °C) and three RH levels (30%, 60%, and 85%). A total of 360 healthy 22-day-old Arbor Acres (AA) broiler chickens with similar body weight were selected and transferred to environmental control chambers, randomly divided into six groups (Groups I, II, III, IV, V, and VI), with six replicates per group and ten chickens per replicate (half male and half female). The pre-trial period lasted 7 days at a temperature of 21 °C and RH of 60%. The formal trial period lasted 14 days. Starting from 29 days of age, the temperature in the environmental control chambers for Groups I, II, and III was adjusted to 26 °C with RH adjusted to 30%, 60%, and 85%, respectively, from 10:00 to 16:00 (6 hours) daily; Groups IV, V, and VI were adjusted to 31 °C with RH adjusted to 30%, 60%, and 85%, respectively. During the remaining time, the temperature and RH in the chambers were maintained the same as during the pre-trial period.

The results showed: 1) The bursa of Fabricius index of broiler chickens in Groups I and V was significantly higher than that in Groups II and VI ($P<0.05$). The bursa of Fabricius index was significantly higher at 30% and 60% RH than at 85% RH ($P<0.05$). 2) On day 7 of the experiment, the serum globulin content of broiler chickens in Group II was significantly higher than that in Groups III, IV, and VI ($P<0.05$). The serum globulin content was significantly higher at 26 °C than at 31 °C ($P<0.05$). 3) On day 1 of the experiment, the serum lysozyme activity of broiler chickens in Group II was significantly higher than that in Groups III, IV, and V ($P<0.05$). The serum lysozyme activity was significantly higher at 60% RH than at 30% and 85% RH ($P<0.05$). On day 7 of the experiment, the serum lysozyme activity of broiler chickens in Group II

was significantly higher than that in Groups I, V, and VI ($P<0.05$). On days 1 and 14 of the experiment, the serum lysozyme activity was significantly higher at 26 °C than at 31 °C ($P<0.05$). 4) On days 1 and 14 of the experiment, the serum IL-1β content of broiler chickens in Group VI was significantly higher than that in Groups I, II, III, and V ($P<0.05$). The serum IL-1β and IL-6 contents were both significantly higher at 31 °C than at 26 °C ($P<0.05$). On days 1, 7, and 14 of the experiment, the serum IL-6 content of broiler chickens in Groups III, IV, V, and VI was significantly higher than that in Groups I and II ($P<0.05$). The serum IL-6 content was significantly higher at 85% RH than at 30% and 60% RH ($P<0.05$).

In conclusion, RH and intermittent partial heat treatment, as well as their interaction, affected the immune function of broiler chickens to varying degrees, with the combination of higher heat and higher humidity (31 °C + 85% RH) having the greatest impact.

Full Text

Effects of Relative Humidity and Intermittent Partial Heat Temperatures on Immune Functions of Broilers

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Abstract

This experiment investigated the effects of relative humidity (RH) and intermittent partial heat temperatures on immune functions in broilers. Using a 2 × 3 factorial design with two partial heat temperature levels (26 and 31 °C) and three RH levels (30%, 60%, and 85%), 360 healthy 22-day-old Arbor Acres (AA) broilers of similar body weight were allocated to six environmental chambers and randomly divided into six groups (Groups I, II, III, IV, V, and VI). Each group contained six replicates of ten birds each (half male and half female). A 7-day pre-test period was maintained at 21 °C and 60% RH, followed by a 14-day trial period. Starting at 29 days of age, Groups I, II, and III were exposed to 26 °C for six hours daily (10:00–16:00) with RH set at 30%, 60%, and 85%, respectively; Groups IV, V, and VI were exposed to 31 °C for the same duration with RH at 30%, 60%, and 85%, respectively. For the remaining 18 hours each day, all groups were maintained at the pre-test conditions (21 °C, 60% RH).

The results showed: (1) The bursa index in Groups I and V was significantly

higher than in Groups II and VI ($P < 0.05$), and bursa indices at 30% and 60% RH were significantly higher than at 85% RH ($P < 0.05$). (2) On day 7, serum globulin content in Group II was significantly higher than in Groups III, IV, and VI ($P < 0.05$), and serum globulin content at 26 °C was significantly higher than at 31 °C ($P < 0.05$). (3) On day 1, serum lysozyme activity in Group II was significantly higher than in Groups III, IV, and V ($P < 0.05$), and activity at 60% RH was significantly higher than at 30% and 85% RH ($P < 0.05$). On day 7, Group II lysozyme activity remained significantly higher than in Groups I, V, and VI ($P < 0.05$). On days 1 and 14, lysozyme activity at 26 °C was significantly higher than at 31 °C ($P < 0.05$). (4) On days 1 and 14, serum IL-1β content in Group VI was significantly higher than in Groups I, II, III, and V ($P < 0.05$), and both IL-1β and IL-6 contents at 31 °C were significantly higher than at 26 °C ($P < 0.05$). On days 1, 7, and 14, serum IL-6 content in Groups III, IV, V, and VI was significantly higher than in Groups I and II ($P < 0.05$), and IL-6 content at 85% RH was significantly higher than at 30% and 60% RH ($P < 0.05$). In conclusion, RH, intermittent partial heat temperatures, and their interaction affect broiler immune function to varying degrees, with the combination of higher temperature and humidity (31 °C + 85% RH) having the greatest impact.

Keywords: relative humidity; intermittent partial heat temperatures; broilers; immune function

Introduction

Relative humidity (RH) is a crucial thermal environmental factor that affects poultry health and production by influencing heat dissipation and thermoregulatory balance. When ambient temperature becomes excessively high, evaporative heat loss becomes the primary cooling mechanism for poultry. However, high RH impairs evaporative cooling capacity, leading to hyperthermia, accelerated respiration, dehydration, and respiratory alkalosis. Most research on RH effects in poultry has focused on physiological indicators and growth performance. Studies have shown that both high (>70% RH) and low (35% RH) humidity affect body temperature and skin temperature in poultry under various high-temperature conditions (29.4, 30.0, 32.0, and 35.0 °C). Yahav et al. reported that broilers aged 4–8 weeks exhibited optimal growth rate and feed intake at 35 °C with 60–65% RH, while Prince et al. and Winn et al. found no significant effect of RH on growth rate or feed intake.

Research on humidity' s impact on poultry immune function remains limited. Wei found that in broilers at 25–26 °C, chronic exposure to high ammonia levels (30 and 70 mg/kg) combined with non-optimal humidity (35% RH and 80% RH) reduced immune function. Intermittent heat stress is common in poultry production, with previous studies demonstrating adverse effects on growth performance, physiological indicators, behavior, thermoregulation, and intestinal

microflora. However, partial heat temperatures (26–32 °C) typically occur intermittently rather than continuously, usually peaking around midday. Therefore, this study investigated the effects of RH and intermittent partial heat temperatures on broiler immune function to provide a theoretical basis for environmental control and refined, comfortable poultry production practices.

1.1 Experimental Animals

Three hundred sixty healthy 22-day-old Arbor Acres (AA) broilers with similar body weight (968 ± 58 g) were randomly divided into six groups (I, II, III, IV, V, and VI), with six replicates per group and ten birds per replicate (half male and half female). The experiment was conducted in environmental chambers at the Changping Experimental Base of the State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, with automatic temperature and humidity control ($±$1 °C, $±7^{2}$ per eight birds. The basal diet was formulated according to NRC (1994) requirements; composition and nutrient levels are shown in Table 1 . Birds had ad libitum access to feed and water throughout the experiment.

1.2 Experimental Design

A 2 × 3 factorial design was employed with two partial heat temperature levels (26 and 31 °C) and three RH levels (30%, 60%, and 85%). Group I: 26 °C + 30% RH; Group II: 26 °C + 60% RH; Group III: 26 °C + 85% RH; Group IV: 31 °C + 30% RH; Group V: 31 °C + 60% RH; Group VI: 31 °C + 85% RH. The pre-test period lasted 7 days at 21 °C and 60% RH. During the 14-day trial period starting at 29 days of age, Groups I, II, and III were exposed to 26 °C for six hours daily (10:00–16:00) with RH at 30%, 60%, and 85%, respectively; Groups IV, V, and VI were exposed to 31 °C with RH at 30%, 60%, and 85%, respectively. For the remaining 18 hours, temperature and RH were maintained at pre-test levels. Temperature and RH were continuously monitored using Testo 174 H mini temperature and humidity data loggers (range: -20 to +70 °C, 0–100% RH; accuracy: $±$0.5 °C, $±$3% RH; resolution: 0.1 °C, 0.1% RH).

1.3.1 Immune Organ Index

On day 14, six broilers per group (one per replicate, three males and three females) were weighed, euthanized by cervical dislocation, and the spleen, thymus, and bursa were excised. After blotting blood with filter paper and removing surface fat and mesentery with surgical scissors, organs were weighed on an electronic balance. The immune organ index was calculated as: immune organ index (g/kg) = immune organ weight (g) / body weight (kg).

1.3.2 Serum Protein, Cytokine Content, and Lysozyme Activity

On days 1, 7, and 14, six broilers per group (one per replicate, three males and three females) were selected for blood collection from the wing vein. Serum

was separated by centrifugation at 3,000 r/min for 10 minutes and stored at -80 °C for analysis. Serum total protein was measured using the Coomassie brilliant blue method, albumin by bromocresol green colorimetry, and globulin content was calculated as the difference. Lysozyme activity was measured by optical assay, and cytokine contents (interleukin-1β [IL-1β] and interleukin-6 [IL-6]) were determined by enzyme-linked immunosorbent assay (ELISA). All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.4 Statistical Analysis

Data were analyzed using the General Linear Model (GLM) procedure in SAS 9.2 software for two-way ANOVA, with Duncan' s multiple range test used for post-hoc comparisons. Results are expressed as means, with $P < 0.05$ considered statistically significant.

2.1 Effects of RH and Intermittent Partial Heat Temperatures on Immune Organ Index

As shown in Table 2 , a significant interaction between temperature and RH affected the bursa index ($P < 0.05$). The bursa index in Groups I and V was significantly higher than in Groups II and VI ($P < 0.05$). RH significantly affected the bursa index ($P < 0.05$), with indices at 30% and 60% RH significantly higher than at 85% RH ($P < 0.05$). Temperature had no significant effect on immune organ indices ($P > 0.05$), though values were numerically higher at 26 °C than at 31 °C.

2.2 Effects of RH and Intermittent Partial Heat Temperatures on Serum Protein Content

Table 3 shows that temperature and RH did not significantly interact to affect serum protein content ($P > 0.05$). However, on day 7, serum globulin content in Group II was significantly higher than in Groups III, IV, and VI ($P < 0.05$). RH had no significant effect on serum protein content ($P > 0.05$). Temperature significantly affected serum globulin content on day 7 ($P < 0.05$), with content at 26 °C significantly higher than at 31 °C ($P < 0.05$).

2.3 Effects of RH and Intermittent Partial Heat Temperatures on Serum Lysozyme Activity

Table 4 reveals significant interactions between temperature and RH on serum lysozyme activity on days 1 and 7 ($P < 0.05$). On day 1, Group II lysozyme activity was significantly higher than in Groups III, IV, and V ($P < 0.05$). On day 7, Group II activity remained significantly higher than in Groups I, V, and VI ($P < 0.05$). Temperature significantly affected lysozyme activity on days 1 and 14 ($P < 0.05$), with activity at 26 °C significantly higher than at 31 °C (P

< 0.05). RH significantly affected lysozyme activity on day 1 ($P < 0.05$), with activity at 60% RH significantly higher than at 30% and 85% RH ($P < 0.05$).

2.4 Effects of RH and Intermittent Partial Heat Temperatures on Serum Cytokine Content

Table 5 shows significant temperature × RH interactions for serum IL-1β content on days 1 and 14, IL-6 content on days 1, 7, and 14, and IL-6 content on day 7 ($P < 0.05$). On days 1 and 14, IL-1β content in Group VI was significantly higher than in Groups I, II, III, and V ($P < 0.05$). On days 1, 7, and 14, IL-6 content in Groups III, IV, V, and VI was significantly higher than in Groups I and II ($P < 0.05$). RH significantly affected IL-6 content on days 1, 7, and 14 ($P < 0.05$), with content at 85% RH significantly higher than at 30% and 60% RH ($P < 0.05$). Temperature significantly affected IL-1β and IL-6 contents on days 1 and 14 ($P < 0.05$), with both cytokines significantly higher at 31 °C than at 26 °C ($P < 0.05$).

3.1 Effects of RH and Intermittent Partial Heat Temperatures on Immune Organ Index

Immune organs are the source of effector cells for humoral and cellular immunity. Their weight and index reflect immune competence and serve as common indicators for evaluating immune status. Few studies have examined humidity effects on immune organ indices. Wei reported that under chronic ammonia and humidity stress at 25–26 °C, low (30% RH) or high (85% RH) humidity did not affect immune organ indices during weeks 1–2, but high humidity significantly reduced thymus and bursa indices by week 3, suggesting that humidity effects increase over time. In this study, a significant temperature × RH interaction affected the bursa index on day 14, with Groups I and V showing significantly higher values than Groups II and VI. RH significantly affected the bursa index, with higher values at 30% and 60% RH than at 85% RH. These results indicate that higher temperature combined with higher humidity adversely affects bursal development. The lack of significant effects on thymus and spleen indices suggests the bursa, as a central immune organ, is more sensitive to humidity than the spleen.

3.2 Effects of RH and Intermittent Partial Heat Temperatures on Serum Protein Content and Lysozyme Activity

Serum total protein and globulin content are important indicators of non-specific humoral immunity. This study found no significant temperature × RH interaction or RH effect on serum protein content. However, temperature significantly affected serum globulin content on day 7, with higher content at 26 °C than at 31 °C. Wei reported no significant humidity effect on serum total protein, albumin, or globulin content. These discrepancies with continuous heat stress

studies may reflect differences in temperature magnitude and treatment pattern (intermittent vs. continuous).

Lysozyme degrades bacterial cell walls, triggering immune responses. As a common immune factor in body fluids and tissues, it plays a vital role in defense and physiological homeostasis. Serum lysozyme activity serves as an indicator of immune function. This study revealed significant temperature × RH interactions on days 1 and 7, with Group II showing highest activity. Temperature significantly affected lysozyme activity on days 1 and 14, with higher activity at 26 °C than at 31 °C. RH significantly affected activity on day 1, with 60% RH producing higher activity than 30% and 85% RH. The diminishing effects over time suggest that birds developed adaptive responses, reducing sensitivity to temperature and humidity.

3.3 Effects of RH and Intermittent Partial Heat Temperatures on Serum Cytokine Content

Cytokines mediate and regulate immune responses, inflammation, hematopoiesis, and tissue repair. IL-1 and IL-6 are key pro-inflammatory cytokines in the initial phase of infection. IL-1β is the primary secreted form in plasma and tissue fluid. This study found significant temperature × RH interactions for IL-1β on days 1 and 14 and IL-6 on days 1, 7, and 14. RH significantly affected IL-6 content on all sampling days, with 85% RH producing higher values than 30% and 60% RH. Temperature significantly affected both cytokines on days 1 and 14, with higher contents at 31 °C than at 26 °C. These results differ from continuous heat stress studies showing elevated IL-1β on day 14 and Wei' s findings of sustained IL-1β and IL-6 increases under humidity stress. Wang reported that acute 35 °C heat stress elevated liver IL-6 content within 0.5 h, peaking at 1.5–2 h before declining to normal by 9–12 h, while continuous 35 °C stress elevated IL-6 from days 1–3 before normalizing by day 5. This "bell-shaped" temporal pattern indicates that cytokine responses vary with stress duration and intensity.

In conclusion, intermittent partial heat treatment, RH, and their interaction affect broiler immune function to varying degrees, with the combination of higher temperature and humidity (31 °C + 85% RH) exerting the greatest impact.

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