Journal of Thermal Biology 70 (2017) 9–14

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

The effect of heat stress on intestinal integrity and *Salmonella* invasion in broiler birds

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ARTICLE INFO

Keywords:
Chickens
Heat stress
Intestinal integrity
Endotoxin
Cytokines
Salmonella

ABSTRACT

The intestinal mucosa works as a barrier to protect the internal environment of the animal from bacteria and bacterial toxins found in the gut lumen. Heat stress may harm this function. Therefore, we designed the current experiment to investigate the effect of heat stress on intestinal integrity, physiological and immunological responses and *Salmonella* invasion in broiler chickens. At 26 days of age, 72 birds were randomly distributed into 3 treatments, with 8 replicates per treatment and 3 birds per replicate. The three treatments were control treatment; kept at thermoneutral environmental conditions (20 ± 2 °C), chronic heat stress treatment (exposed to 30 ± 2 °C; 24 h/day) and acute heat stress treatment (exposed to 35 ± 2 °C from 09:00 to 13:00 and kept at 20 ± 1 °C from 13:00 to 09:00). The heat stress exposure was conducted for 10 successive days. Compared with the control treatment, birds subject to chronic and acute heat stress had reduced ($P < 0.05$) body weight and body gain and increased ($P < 0.05$) feed conversion ratio. However, feed intake and mortality rate were only increased ($P < 0.05$) in the acute heat stress treatment. Rectal temperature and Δ rectal temperature (°C/h) increased ($P < 0.05$) sharply during the first 2 days of exposure followed by gradual decreases until a plateau was achieved. Heat-stressed birds had increased ($P < 0.05$) serum concentrations of corticosterone, endotoxin lipopolysaccharide and the systemic inflammatory cytokine: TNF-α and IL-2, as well as a higher ($P < 0.05$) prevalence of *Salmonella spp*. in meat and livers, as compared with control treatment. It can be concluded that heat stress impaired intestinal integrity which resulted in increased intestinal permeability to endotoxin, translocation of intestinal pathogens (*Salmonella spp*.) and serum inflammatory cytokines. Therefore, avoiding thermal dysfunction of intestinal barrier is a significant factor in maintaining welfare, immune status and meat safety of broiler birds.

1. Introduction

Heat stress is a critical problem of major concern due to the global warming (Hansen et al., 2010). It negatively impacts the fast growing broiler birds compared with the slow growing layers, particularly during hot climates and during the summer season in temperate climates (Altan et al., 2003). High ambient temperature has been shown to influence broiler physiology by inducing multiple physiological disturbances, such as systemic immune dysregulation, endocrine and electrolyte disorders which result in poor growth and increased mortality (Quinteiro-Filho et al., 2012; Abdelqader and Al-Fataftah, 2014). In addition, it has been reported that heat stress negatively affects intestinal development and functions (Garriga et al., 2006), especially the integrity of the intestinal epithelium (Pearce et al., 2013). The intestinal epithelium plays important roles in the digestion and absorption of nutrients and acts as a barrier between the internal and external environments. The intestinal mucosa is continuously exposed to a heavy load of antigen molecules from ingested food and microorganisms, such as resident and invasive bacteria and viruses (Keita and Soderholm, 2010). The effectiveness of the intestinal barrier is essential in maintaining the health and livability of living organisms. Dysfunction of this barrier increased intestinal permeability which facilitate the passage of antigens and bacteria from the gut lumen into bloodstream leading to pathological conditions (Keita and Soderholm, 2010). Environmental stressors have also been shown to induce the intestinal colonization of enteric pathogens in poultry, which facilitates horizontal transmission and, consequently, increases the contamination of poultry products (Burkholder et al., 2008; Abdelqader and Al-Fataftah, 2016). Furthermore, stress-induced impairment of the integrity of the intestinal epithelium reduces the efficacy of the birds' innate protective mechanisms and may increase the potential for intestinal inflammation (Burkholder et al., 2008). Environmental stressors caused local and

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http://dx.doi.org/10.1016/j.jtherbio.2017.10.015
Received 17 August 2017; Received in revised form 22 October 2017; Accepted 24 October 2017
Available online 31 October 2017

systemic inflammation in human and rats models (Hall et al., 2001; Leon, 2007).

Recently, the gut has been reported to be negatively affected by heat stress manifested mainly as intestinal mucosa damages, impairment of intestinal epithelium and immune suppression (Pearce et al., 2013; Abdelqader et al., 2017). Diverse birds physiological and immunological responses are based on the duration and magnitude of heat stress which make it difficult to study the impacts of heat stress on poor intestinal functions in isolation. Broiler birds in hot regions encounter either a chronic exposure to high ambient temperature or a sudden raise in ambient temperature manifested as heat waves (Abdelqader and Al-Fataftah, 2014). Thus, this study was designed to investigate the effects of chronic and acute heat stress on performance parameters, intestinal integrity, physiological and immunological responses and *Salmonella* invasion in broiler birds.

2. Materials and method

2.1. Birds and experimental design

Birds care and handling were in compliance with the EU legislation for the protection of animals used for scientific purposes (Directive number 2010/63/EU). The experimental protocols was also approved by the Scientific Research Council of the University of Jordan. This experiment was conducted at the Animal Environmental Physiology lab located at the Faculty of Agriculture, the University of Jordan. One-day old Hubbard classic broiler chicks (initial body weight; 45 g) were obtained from a commercial hatchery; kept in cages (dimensions: 188 cm × 82 cm × 68 cm) with wire mesh floor and reared under routine commercial management practices. Birds were provided with optimum rearing temperature according to the Hubbard strain guideline and given ad libitum feed and water. At 26 days of age, seventy-two birds were randomly distributed into 3 treatments, with 8 pen replicates (3 bird/pen; 24 birds/treatment). The three treatments were: control treatment; kept at thermoneutral environmental conditions (20 ± 2 °C; 42–66% RH), chronic heat stress treatment; kept in an environmentally-controlled chamber and continuously exposed to a high ambient temperature (30 ± 2 °C; 33–53% RH; 24 h/day for 10 successive days), and acute heat stress treatment; exposed daily to high acute ambient temperature (35 ± 2 °C; 33–38% RH) from 09:00 to 13:00 in an environmentally-controlled chamber and returned back to the thermoneutral conditions (20 ± 2 °C) from 13:00 to 09:00. The heat exposure protocol was conducted for 10 successive days (from 26 to 35 days of age). The 20 °C is considered as a thermoneutral environment for chickens at this age (Charles, 2002; Sturkie, 1965). The chronic and acute models of heat exposure were created, respectively, to simulate the chronic heat stress and the sudden heat waves that occur in the nature.

2.2. Performance parameters

Total daily feed intake/replicate was measured every morning at 08:00 a.m. from day 26–35 of age. Average daily feed intake was calculated at the end of the experiment. Body weights were measured on days 0, 7 and 10 of the experiment to calculate average daily gain. Feed conversion ratio was corrected for number of birds per pen and calculated on the basis of kg of feed consumed per kg of live body weight gain. Mortality was recorded on a daily basis and feed conversion ratio was corrected for mortality or the number of birds slaughtered for sampling.

2.3. Rectal temperature

Rectal temperature was measured on a daily basis before the start of heat exposure at 09:00 for all treatment groups, and then at hourly intervals. Average daily rectal temperature was calculated from rectal temperatures measured during the heat exposure periods between 9:00 and 13:00. Rectal temperature was measured by a digital thermometers (± 0.01 °C accuracy) connected to a very fine probe that was inserted for a distance of maximum 5 cm inside the rectum of each bird. Rate of increase in rectal temperature (Δ °C/h) is a calculated parameter was determined every day by calculating the difference in rectal temperature before the start of heat exposure and at the end of exposure divided by the time between them in hours.

All birds were given a five-day training period (pre-heat exposure period; day 21–25 of age) before starting of the experiment. The aim of this period was to get the birds used to the experimental conditions and the measurement practice. During the pre-heat exposure period, the rectal temperature was measured at hourly intervals from 09:00 to 13:00 for all birds.

2.4. Blood sampling

Blood collection was conducted twice during the experimental period, on day 3 and day 10 of the experiment. A total of 24 blood samples were collected from 24 birds, 8 from each treatment, every time. Blood samples were collected from the jugular vein in heparinized tubes and immediately centrifuged at 115 g for 15 min to obtain the serum. The serum was further used for the analysis of corticosterone hormone, endotoxin, and cytokines.

2.4.1. Serum corticosterone determination

Corticosterone concentration in the serum was measured using an enzyme-linked immunoassay commercial ELISA kit (Corticosterone ELISA kit; EIA-4164; DRG Instruments GmbH, Germany) the procedure was followed as provided by the supplier.

2.4.2. Serum cytokines

Serum cytokines; IL-2 and TNF-α, were measured using a commercial available kits specific for chicken (ELISA kits, CUSABIO BIOTECH CO., LTD, China). Levels of IL-2 and TNF-α in serum samples were measured according to manufacturer's instructions.

2.4.3. Intestinal permeability

The permeability of the small intestine was evaluated by measuring the levels of serum endotoxin (lipopolysccharied; LPS). Serum endotoxin levels was measured using a commercial available kit [Endotoxin Detection System, limulus amebocyte lysate (LAL) QCL-1000, quantitative chromogenic LAL, Lonza, BioWhittaker, Walkersville, MD, USA].

2.5. Meat and liver safety

To investigate the presence or absence of *Salmonella* spp. in the meat or liver, tissue samples were taken from 8 birds from each experimental treatments on day 3 and day 10. For isolation and identification of *salmonella*, the samples were diluted in buffer peptone water (1:10) and homogenized using stomacher and then incubated at 37 °C for 16 − 20 h. One ml of incubated pre-enrichment homogenate was transferred into 10 ml Selenite cystine broth, and incubated at 37 °C for 24 h. One loopful from enriched broth was streaked onto plates of Xylose lysine deoxycholate agar and incubated at 37 °C for 24 h. The plates were examined for the presence of typical colonies of *Salmonella* spp. (red with black center). The presence or absence of *Salmonella* colonies was used to calculate the prevalence % in each cultured sample.

2.6. Statistical analyses

The Statistical Analysis System (SAS Institute, 2010, Version 9.1.3) was used to conduct all statistical analysis. Data that included sampling at 2 different days were analyzed with a linear mixed model that