Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

e-ISSN: 1806-9061 2024 / v.26 / n.3 / 001-012

http://dx.doi.org/10.1590/1806-9061-2024-1915

Original Article

Effects of Chronic and Acute Heat Stress on the Cardiac Expression of Antioxidative and Anti-Inflammatory Genes in Chicks

■Author(s)

Bastaki NK[I] https://orcid.org/0000-0003-4394-0126
Almomen JZ[I] https://orcid.org/0009-0004-9090-2119
Albarjas TA[I] https://orcid.org/0009-0006-4299-8472

[I] Department of Biological Science, Faculty of Science, Kuwait University, P.O. Box 5969, Safat, Kuwait City 13060, Kuwait.

■Mail Address

Corresponding author e-mail address
Nasmah Bastaki
Department of Biological Science, Faculty of Science, Kuwait University, P.O. Box 5969, Safat, Kuwait City 13060, Kuwait.
Phone: +965-50124010
Email: nasmah.bastaki@ku.edu.kw

■Keywords

Heat stress; Oxidative stress; Inflammation; Chicks; Hearts; Gene-expression.

Section editor: Ramon Malheiros

Submitted: 26/February/2024
Approved: 02/August/2024

ABSTRACT

Heat stress can affect several biological pathways. This study aimed to compare the effects of chronic and acute heat stress on the oxidative status and inflammatory responses of chick's hearts. Chronic and acute heat stress were induced in chicks, heart tissues were examined for morphological changes, and gene expression was analyzed in heart samples. Our results showed that prolonged heat exposure caused a dramatic reduction in chicks body weight, increased lesions, and ruptured cardiac muscle fibers in the hearts, confirming that chronic heat stress damages heart tissues and causes inflammation. Our gene expression results confirmed that heat stress induces oxidative stress and inflammation in the hearts of chicks, and this is evidenced by changes in the expression of NRF2 and CAT as antioxidant factors, NFκB and LITAF as anti-inflammatory factors, and changes in the expression of Leptin as an activator of Reactive Oxygen Species production and induction of proinflammatory factors. Our study also showed that the induction of anti-inflammatory and antioxidant genes was greater upon exposure to chronic heat stress than acute heat stress. These findings confirm that chickens generally tolerate chronic heat stress better than acute heat stress.

INTRODUCTION

Chickens are more sensitive to heat stress than mammals because of their feathers and the lack of sweat glands, making the negative impact of heat stress a significant concern for poultry production (Lara & Rostagno, 2013; Saeed *et al.*, 2019; Cândido *et al.*, 2020; Hirakawa *et al.*, 2020; Nawaz *et al.*, 2021). Heat stress affects chicken physiology, increasing heart rate, blood flow, and internal temperature, and decreasing food intake, growth rates, and egg production (Cervantes *et al.*, 2016; Goel, 2021; Ahmad *et al.*, 2022).

Heat stress can be categorized into two types based on the duration of exposure: acute (short-term exposure) and chronic (long-term exposure) (Ghulam Mohyuddin *et al.*, 2022). Chronic heat stress affects animal physiology and behavior, but is generally tolerated by animals and does not lead to death (Morera *et al.*, 2012; Goel, 2021). Acute heat stress, however, can result in hyperthermia, significant damage to organs, and chicken death (Lan *et al.*, 2016; Adu-Asiamah *et al.*, 2021).

Recent studies have demonstrated that heat stress induces oxidative stress through the overproduction of Reactive Oxygen Species (ROS). Overproduction of ROS causes damage to cells and organs by modifying cellular proteins and lipids, and damaging nucleic acids (Sies *et al.*, 2017; Emami *et al.*, 2020; Qixiang Miao *et al.*, 2020; Liu *et al.*, 2021; Alva *et al.*, 2023). One of the first defense mechanisms

against oxidative stress is the expression of detoxifying enzymes such as superoxide dismutase (*SOD*), catalase (*CAT*), and guaiacol peroxidase (*POD*). These enzymes scavenge excess ROS inside the cells (Sies *et al.*, 2017; Liu *et al.*, 2021). One of the crucial regulators of the redox homeostasis under oxidative stress is the activity of the Nuclear factor erythroid 2-related factor 2 (*NRF2*). *NRF2* is a transcription factor that is a master regulator of antioxidant defense mechanisms under oxidative stress. It binds to a conserved antioxidant response element of target genes and promotes the activity of antioxidant enzymes, such as *SOD* and *CAT* (J. F. Zhang *et al.*, 2018; Surai *et al.*, 2021; Tang *et al.*, 2021; Tang *et al.*, 2022).

In addition to oxidative stress, heat stress causes an imbalance in the immune system that triggers inflammation (Abdelnour *et al.*, 2019; Most & Yates, 2021; E. Liu *et al.*, 2022). Several studies have demonstrated that heat stress increases the inflammatory components of the immune system. For example, rats exposed to a few weeks of heat stress showed more leukocytes in the spleen than control rats *(Song et al.*, 2019). Broiler chickens exposed to heat stress for 14 days showed severe damage to the proliferation and differentiation of the immune system in lymphoid tissues (Hirakawa *et al.*, 2020). Upon exposure to heat stress, immune homeostasis must be maintained in order to avoid extreme inflammation and autoimmune diseases (Horwitz *et al.*, 2019; Song *et al.*, 2019). One mechanism by which cells adapt to overcome inflammation is the activation of the Nuclear Factor Kappa B (*NF-κB*) and Tumor Necrosis Factor Alpha (*TNF-* α) pathways (Abdelnour *et al.*, 2019; Daghero *et al.*, 2022; Gao *et al.*, 2022). *NF-κB* is a transcription factor closely related to *NRF2* and a master regulator of inflammation and immune homeostasis (Mitchell & Carmody, 2018). *NF-κB* controls inflammation by activating proinflammatory cytokines such as tumor necrosis factor-α (*TNF-*α)/lipopolysaccharide-induced tumor necrosis factor (*TNF*)-alpha factor (*LITAF*), which consequently activates innate and adaptive immune responses (Mitchell & Carmody, 2018; Del Vesco *et al.*, 2020; Surai *et al.*, 2021).

Leptin is a hormone derived from adipose tissue that plays a major role in energy homeostasis and appetite regulation (Dridi *et al.*, 2008; Laursen *et al.*, 2017; Caruso *et al.*, 2023). Some studies have shown that heat stress increases *Leptin* production. For example, one study showed that chronic heat stress improves *Leptin* signaling in adipose, muscle, and liver tissues in mice (Morera *et al.*, 2012). Acute heat stress has been shown to upregulate hepatic leptin in broiler chickens (Dridi *et al.*, 2008). Another study showed that the expression of *Leptin* was not affected by exposure to chronic heat stress in the adipose tissues of pigs (Cervantes *et al.*, 2016).

In the immune system, *Leptin* stimulates the production of proinflammatory cytokines, and *Leptin* production increases during acute infection and inflammation (Bruun *et al.*, 2002; Sánchez-Margalet *et al.*, 2003; La Cava & Matarese, 2004; Abella *et al.*, 2017). Moreover, *Leptin* is potentially an activator of ROS production, as it increases the generation and accumulation of ROS in cells (Yamagishi *et al.*, 2001; Koh *et al.*, 2008; Mahbouli *et al.*, 2017; Mourmoura *et al.*, 2022). The role of *Leptin* in the cardiovascular system remains uncertain; however, various studies suggest that *Leptin* may be needed for the pathogenesis of chronic inflammation in the heart, as it affects the production of proinflammatory cytokines and oxidative stress, Thus, *Leptin* can be used as a biomarker for heart failure (Schulze & Kratzsch, 2005; Poetsch *et al.*, 2020; Vasamsetti *et al.*, 2023).

In the present study, chronic and acute heat stress were induced in chicks, and gene expression was used to monitor the effect of heat on the progression of oxidation and inflammation in the heart. Five genes were selected for this study: *NRF2* and *CAT* as antioxidant factors, *NFκB* and *LITAF* as anti-inflammatory factors, and *Leptin* as an activator of ROS production and inducer of proinflammatory factors. To the best of our knowledge, this is the first study to monitor *Leptin* expression in chicken hearts after exposure to heat stress.

MATERIALS AND METHODS

Ethical approval

The experiment procedures of this study were approved by the Ethics Committee on Animal Use at Kuwait University and the Ethics Committee for the Use of Laboratory Animals (DBS/IRB(ECULA)20-005 (Bastaki *et al.*, 2022 and Bastaki *et al.*, 2023).

Animal Source for the Study

In the present study, 75 two-week-old chicks (*Gallus gallus domesticus*) were purchased from local farms in Kuwait. Forty chicks were exposed to chronic heat stress and thirty five were exposed to acute heat stress. Chicks in all experiments had free access to food and water.

Distribution and numbers of the chicks in this study

Chronic heat-stress experiment

Two-week-old chicks were randomly divided into eight groups with five replicates each, as follows: control group 1 (indoors for 1 week), control group 2 (indoors for 2 weeks), control group 3 (indoors for 3 weeks), control group 4 (indoors for 4 weeks), heat-stress group 1 (outdoors for 1 week), heat-stress group 2 (outdoors for 2 weeks), heat stress group 3 (outdoors for 3 weeks), and heat-stress group 4 (outdoors for 4 weeks). The control groups were kept indoors at a constant temperature of 25 °C, and the heat-treated groups were kept outdoors, where the temperature fluctuated according to the outside temperature during the summer season in Kuwait (35–50 °C) (Figure 1). Temperatures were recorded three times per day (morning, afternoon, and evening) (Figure 1). The control groups were euthanized indoors, while the heat-treated groups were euthanized outside at noon with direct sun exposure. Immediately after death, heart samples were collected and processed for RNA extraction and histological staining.

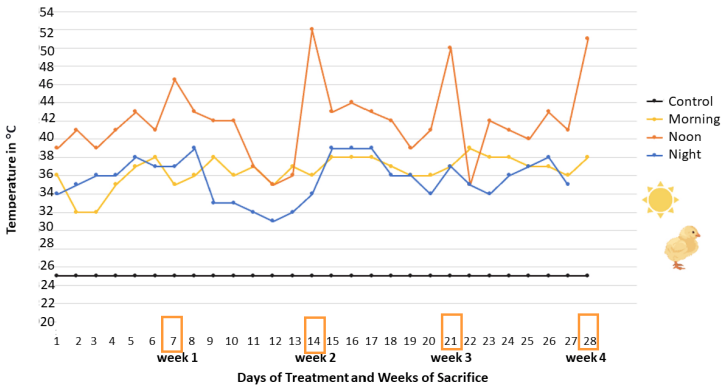

Figure 1 – Temperature record for the induction of chronic heat stress.

Acute heat-stress experiment

Two-week-old chicks were randomly divided into seven groups with five replicates each, as follows: control group (no heat treatment), group 1 (45 min of heat treatment), group 2 (1 h and 30 min of heat treatment), group 3 (2 h and 15 min of heat treatment), group 4 (3 h of heat treatment), group 5 (3 h and 45 min of heat treatment), and group 6 (4 h and 30 min of heat treatment). Chicks in the heat-treated groups were placed in a temperature-controlled growth chamber at a constant temperature of 45 °C. The control group was euthanized indoors at 25 °C. Immediately after death, heart samples were collected and processed for RNA extraction and histological staining.

Histological staining of the heart

Heart samples for the control and heat-treated chicks were processed for histological staining with Meyer's hematoxylin (MHS16, Sigma –Aldrich) and eosin (102439, Sigma-Aldrich) as recently reported in the literature (Bastaki *et al.*, 2022).

Quantitative Real-Time PCR

RNA was extracted from the heart samples using TRIzol reagent according to the manufacturer's protocol (15596018, Invitrogen, Waltham, MA, USA). A cDNA reverse transcription kit was used to synthesize cDNA samples according to the manufacturer's protocol (4368814, Applied Biosystems, Waltham, MA, USA). Quantitative real-time PCR was performed using a Bio-Rad CFX96 Real-Time system (C1000 Touch Thermal Cycle, Bio-Rad, Singapore). PCR reactions were performed using PowerUp SYBR Green Master-Mix 2X (A25779, Applied Biosystems). The primers used in the qRT-PCR are listed in Table 1. Genes were amplified in duplicate, and each PCR was run at least three times.

Table 1 – Description of all primers for the genes used for RT-qPCR analysis.

Gene	Gene Full Name	Primer Sequence (5'–3')	Accession Number
CAT	Catalase	F: ACACAGCATTGCCCTCGATT R:AGGTCCTCAAATGCACCAC	NM_001031215.2
LITAF	lipopolysaccharide induced TNF factor	F:TTGGCCCCCAGTGTTATCAG R:CCGGTGATGCACAGTAACCT	XM_040647309.1
NF-kB	nuclear factor kappa B subunit 1	F:TCAACGCAGGACCTAAAGCAT R:GCAGATAGCCAAGTTCAGGATG	NM_205134.1
LEP	Leptin	F:GCTGCCTTTGTGCTCTGCTAT R: TGAGGGTTCGGCTCAGAC	KT_970642.1
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	F: CACACAGAAGACGGTGGATG R: CAGCTCAGGGATGACTTTCC	NM_204305.2
B-Actin	Beta-Actin	F: ACCCCAAAGCCAACAGA R: CCAGAGTCCATCACAATACC	NM_205518.2
RPL5	Ribosomal Protein L5	F:AATATAACGCCTGATGGGATGG R:CTTGACTTCTCTCTTGGGTTTCT	NM_204581.5

The PCR conditions were 50 °C for 2 min, then 40 cycles of 95 °C for 2 min, 95 °C for 15 s, 57 °C for 15 s, and 72 °C for 1 min. Melting analysis for primer specificity was set at 65 °C for 5 s and 95 °C for 5 s.

Statistical analysis of target genes

Statistical analysis was performed as described by Bastaki *et al.* (2023). In summary, the Ct value was computed using Bio-Rad CFX96 software to quantify the mRNA for each gene, and the average Ct values were calculated for each sample group. Values were normalized using *GAPDH* as an endogenous control. The mRNA quantity was calculated as the ΔCt value (Ct target gene–Ct reference gene *GAPDH*) for each gene. The normalized relative expression ΔΔCt (ΔCt heat-treated sample – ΔCt control sample) was calculated for each gene. The fold-change $2^{-\Delta\Delta Ct}$ was obtained for each gene. Finally, statistical analysis was conducted using one-way ANOVA and two-way ANOVA for acute and chronic heat stress, respectively. GraphPad Prism version 9 (GraphPad Software Inc., San Diego, CA, USA) was used to obtain the *p* value and standard deviation for the expression of each gene. Statistical significance was set at * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$. Data are presented as means ± standard deviation (SD).

RESULTS

Effects of heat stress on growth performance of chicks

The chicks were weighted prior to euthanasia. Weights varied among the chicks exposed to chronic heat stress (Figure 2). The average weights were 27.8 g, 46.6 g, 50.4 g, and 105.6 g in the control 1 (14 days old), control 2 (21 days old), control 3 (28-days-old), and control 4 (35 days old) groups, respectively. The weight increase in the control groups was expected, as the chicks grew weekly. However, the weights of chicks placed outdoors and exposed to chronic heat stress did not increase normally; the average weights were 25.5 g, 33.5 g, 41.5 g, and 42.2 g after one week (14 days old), two weeks (21 days old), three weeks (28 days old), and four weeks (35 days old) of heat stress, respectively. The acute heat stress experiment was conducted in one day on 14-day-old chicks, and the average weights were similar among groups, ranging between 27.8 g and 32.0 g. These results indicate that chronic heat stress affected the feed intake of chicks, resulting in reduced weight gain and growth performance.

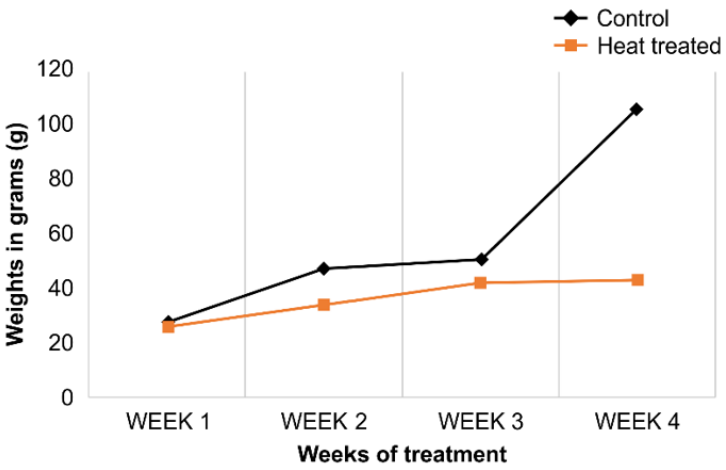

Figure 2 – Average weights of chicks prior to euthanasia in the chronic heat stress groups.

Histological staining of chicken hearts after exposure to chronic heat stress

Histological staining of heart tissue was compared between control chicks and chicks exposed to chronic heat for three and four weeks (Figure 3). Control group 3 (28 days old) and control group 4 (35 days old) had clear and regular cardiac muscle fibers (Figure 3a and 3c). In comparison, heart samples from chicks exposed to chronic heat stress for three (28 days old; Figure 3b) and for four weeks (35-days-old; Figure 3d) showed clear lesions and spaces with fragmented and ruptured cardiac muscle fibers, suggesting inflammation of heart cells and tissue. The results clearly demonstrate that exposure to heat stress for a prolonged period induces heart damage.

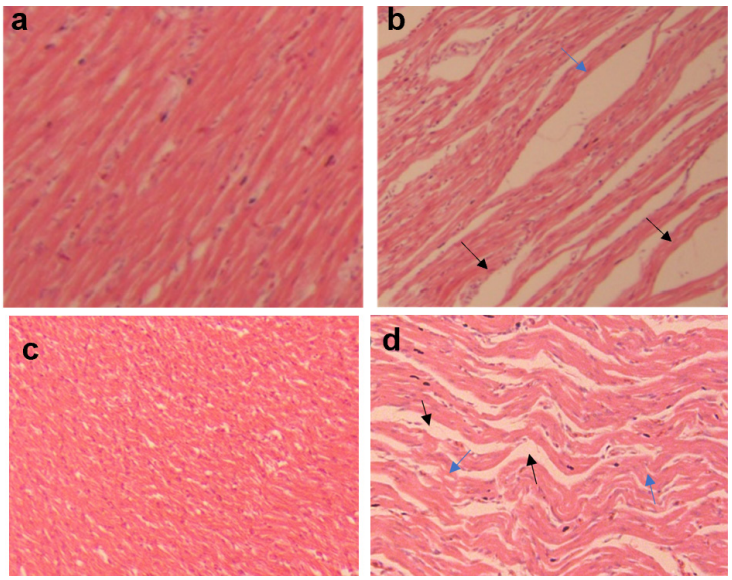

Figure 3 – H&E stained heart tissue sections (x 200) of control (a, c) and heat-stressed hearts (b,d). Arrows indicate lesions and spaces with fragmented and ruptured cardiac muscle fibers.

Effects of chronic and acute heat stress on antioxidative gene expression

To monitor the effect of heat stress on the oxidative status of the heart, two antioxidative factors were selected for gene expression analysis: *NRF2* as a transcription factor of the antioxidant defense mechanism, and *CAT* as an antioxidant enzyme promoted by *NRF2*.

Upon exposure to chronic heat stress for one to four weeks, both genes showed similar expression patterns. After the first week of heat exposure, expression was downregulated in the heat-stressed hearts compared to the control hearts; however, expression of both genes was upregulated after the second week of heat exposure compared with the control chicks (Figure 4a and b), with a significant value ($p \leq 0.05$) detected only for *CAT* and not for *NRF2*. Both genes were slightly downregulated in the third and fourth weeks of chronic heat treatment.

Upon exposure to acute heat stress, there were fluctuations in the expression of *NRF2* and *CAT*, but both showed a similar gene expression pattern. Expression was downregulated after the first period of heat exposure, and then started to increase gradually until it peaked after two hours of exposure. After the two hours, expression fluctuated again, reducing and then increasing (Figures 4c and d).

Our results demonstrated similar gene expression patterns for *NRF2* and *CAT* under chronic and acute heat stress.

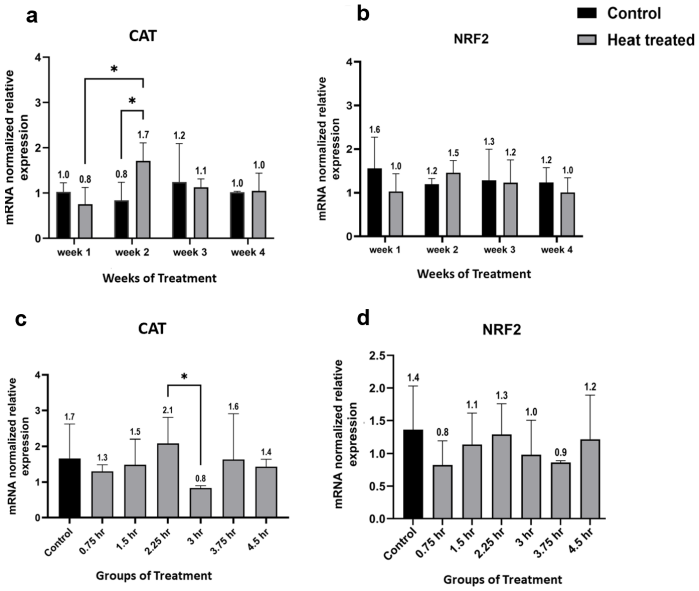

Figure 4 – The fold change differences (2^ΔΔCt) of the relative normalized expression for *CAT* (a and c) and *NRF2* (b and d) in the control and heat-treated chicks after exposure to chronic heat stress for one to four weeks, or acute heat stress for 45 min to 4 hr and 30 min (n = 3 in each group, * $p \leq 0.05$). Data are presented as means ± SD.

Effects of chronic and acute heat stress on anti-inflammatory gene expression

To monitor the effect of heat stress on the inflammation status of the heart, two anti-inflammatory factors were selected for gene expression analysis: *NF-kB* as a transcription factor of inflammation and immune homeostasis, and *LITAF* as proinflammatory cytokine controlled by *NF-kB.*

NF-kB expression was significantly upregulated ($p \leq 0.0001$) after the first week of chronic heat exposure in the hearts of heat-stressed chicks compared with that of control hearts. Expression was dramatically reduced after the second week of heat exposure ($p \leq 0.001$), but it was still slightly higher than in the controls. After the 3rd and 4th week of heat exposure, *NF-kB* expression was downregulated in the hearts of heat-stressed chicks compared control hearts (Figure 5a), and significantly lower than that of the first week's heat exposure ($p \leq 0.0001$). As for *LITAF,* its expression was upregulated in the hearts of heat-stressed chicks after the 2nd and 3rd weeks of heat stress; however, after the 1st and 4th weeks, it was downregulated (Figure 5b). No significant values were detected for *LITAF* in the chronic heat stress groups.

Upon exposure to acute heat stress, expression of *NF-kB* and *LITAF* was downregulated at different time points from the beginning to the endpoint of heat exposure (Figure 5 c and d). A significant decrease ($p \leq 0.05$) was detected for *LITAF* in all acute heat treatments, but only at 3 and 4.5 hours for *NF-kB*.

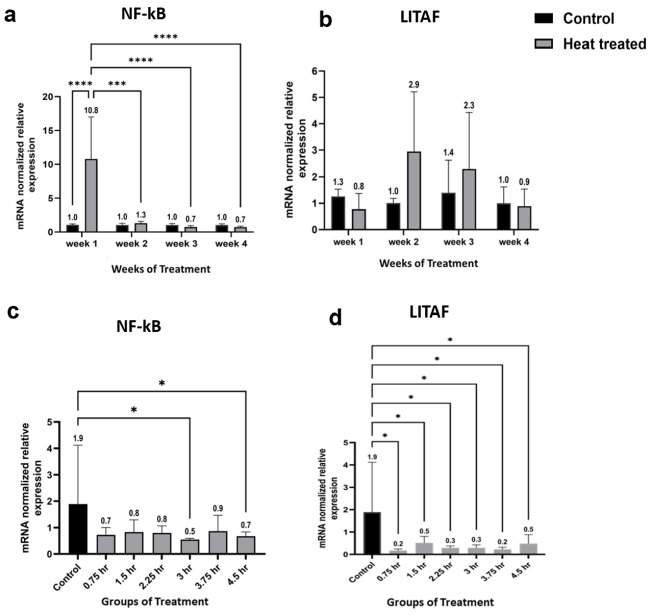

Figure 5 – The fold change differences (2^ΔΔCt) of the relative normalized expression for *NFKB* (a and c) and *LITAF* (b and d) in the control and heat-treated chicks after exposure to chronic heat stress for one to four weeks or acute heat stress for 45 min to 4 hr and 30 min (n = 3 in each group, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$). Data are presented as means ± SD.

Effects of chronic and acute heat stress on Leptin gene expression

Leptin expression was significantly upregulated ($p \leq 0.05$) upon the first week of heat exposure in the chronically heat-stressed hearts compared with that in the control hearts . In the heat-stressed hearts, *Leptin* expression was significantly diminished ($p \leq 0.01$) after the 2nd and 3rd weeks of heat exposure, and after the 4th week, but the expression was slightly higher than in the control hearts (Figure 6a).

Leptin expression was lower in the hearts of chicks exposed to acute heat stress than in the control hearts throughout the examined time, with slight variation in expression. Expression was highest after 45 min of heat exposure than in the rest of the examined time periods (Figure 6b), but significant decreases ($p \leq 0.05$) were observed only at 1.5 and 4.5 hours.

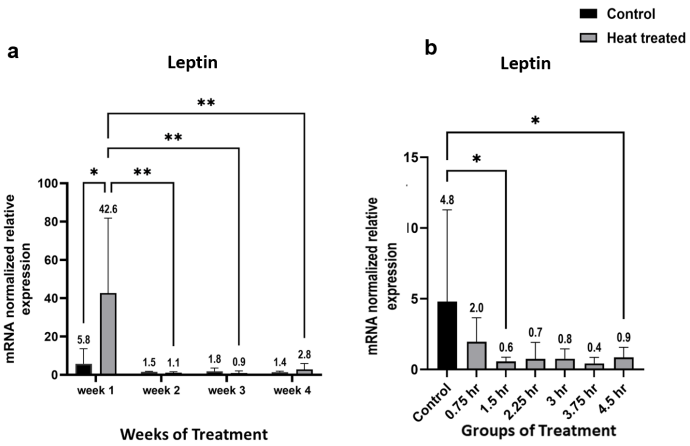

Figure 6 – The fold change differences (2^ΔΔCt) of the relative normalized expression for *Leptin* in the control and heat-treated chicks after exposure to chronic heat stress for one to four weeks (a) or acute heat-stress for 45 min to 4 hr and 30 min (b) (n = 3 in each group, * $p \leq 0.05$, ** $p \leq 0.01$). Data are presented as means ± SD.

DISCUSSION

Chickens are homoeothermic and lack sweat glands; therefore, they are more susceptible to heat stress than other animals (Murugesan *et al.*, 2017; Cândido *et al.*, 2020). The body temperature of a newly hatched chick is approximately 39.7 °C, and this temperature increases every day until it becomes stable at three weeks post-hatch. The body temperature of an adult chicken is approximately maintained between 40.0 and 40.5 °C (Donald & William, 2002; Saeed *et al.*, 2019). In practice, the best environmental temperature for chicks to maintain optimal body temperature is between 24 and 26°C. Above this ideal range, the air temperature may induce a state of heat stress known as hyperthermia, which compromises normal functions of many organs and leads to high mortality rates (Hamissou Maman *et al.*, 2019; Brugaletta *et al.*, 2022; Burhans *et al.*, 2022).

Oxidative stress and inflammatory responses are two major causes of heat stress. Under normal conditions, all aerobic organisms maintain a redox balance between the production of ROS through metabolism and the reduction of ROS through antioxidants. Heat stress interrupts this balance by increasing the production of ROS and decreasing antioxidant activity, causing oxidative stress (Birben *et al.*, 2012; Akbarian *et al.*, 2016; Jacobs *et al.*, 2020). Heat stress also increases the inflammatory components of the immune system and causes overproduction of inflammatory cytokines (Min *et al.*, 2016; Barnes *et al.*, 2021; Most & Yates, 2021; Park *et al.*, 2021).

The objective of the present study was to examine oxidative and inflammatory responses in heart samples of chicks exposed to chronic and acute heat stress. Chicks in each group in the chronic and acute experiments were examined for behavioral changes, including respiratory rate, heart rate, and feeding intake. The weights of the chicks in the acute heat experiment were similar between the control and heat-treated groups; however, the weights were different between the control and heat-treated chicks in the chronic heat experiment. After the 1st week of heat treatment, the difference in averages between the control and treated groups was 2.3 g (Figure 2). The difference became more noticeable in the subsequent weeks, rising to 13 g after the 2nd week, 8.9 g after the 3rd week, and 63.4 after the 4th week of heat treatment. The results demonstrated that prolonged heat exposure decreases feed intake, causing a dramatic reduction in chick weight. Our results matched those of other studies that reported heat stress inhibiting the growth performance of broilers, and prolonged exposure to chronic heat not resulting in a higher mortality compared with acute heat (Quinteiro-Filho *et al.*, 2010; Y.-L. Liu *et al.,* 2022). In both experiments, one to two deaths occurred in each group for the control and heat-treated groups.

Histological staining was used to examine the effect of chronic heat exposure on heart tissue. Our results on the histopathological changes in heart samples exposed to chronic heat stress agreed with those from previous studies (Tang *et al.*, 2014; Nasrolahi *et al.*, 2020). The lesions and ruptured cardiac muscle fibers observed in our study also matched previously published results on chicken hearts exposed to cold stress (Zhao *et al.*, 2013; Wei *et al.*, 2018). Histopathological changes due to chronic heat have also been reported in tissues

such as intestines (Yu *et al.*, 2010; Abdel-Rahman *et al.*, 2022; W.-C. Liu *et al.*, 2022), muscles (Ohira *et al.*, 2017), liver (Ma *et al.*, 2022; Tang *et al.*, 2022), lungs (Lin *et al.*, 2019; Wu *et al.*, 2023) and kidneys (Aengwanich & Simaraks, 2004; Goto *et al.*, 2022). These studies and ours clearly demonstrate that heat stress has a dramatic effect on tissue and organ stability and functions, and can lead to organ failure.

Before the quantitative RT-PCR analysis of the antioxidative and anti-inflammatory genes in the hearts, the best reference gene as a normalizer for the experiment was tested and selected accordingly. Three endogenous reference genes were tested in this study: *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase), *B-actin* (beta-actin), and *RPL5* (ribosomal protein L5) (Table 1). Our results showed that *GAPDH* was the best gene normalizer for heart samples, as it had the lowest and most consistent Ct values in both control and heat-treated chicks (data not shown). Therefore, *GAPDH* was chosen as the gene normalizer for the quantitative RT-PCR in this study, which was consistent with our recently reported study using retinal samples (Bastaki *et al.*, 2023).

Our results showed that the antioxidant factors *NRF2* and *CAT* had similar patterns of expression under chronic and acute heat stress. Under chronic stress, expression was upregulated only after the 2nd week of heat treatment (Figure 4a and b). The temperature recorded in the afternoon was 46 °C after the 1st week of heat treatment and 52°C after the 2nd week of heat treatment. Therefore, it is possible that the rise in temperature in the 2nd week caused an increase in oxidative stress, which led to increased expression of the antioxidative factors *NRF2* and *CAT* in the hearts of heat-stressed chicks. However, after the 3rd and 4th week of heat treatment, the outdoor temperature was similar to that of the 2nd week, but the expressions of *NRF2* and *CAT* were slightly reduced. The expression of *NRF2* and *CAT* under acute heat stress was similar and fluctuated across the different treatment groups. The highest expression of *NRF2* and *CAT* was observed in the 3rd week treatment group (2 h 15 min of heat exposure).

Our results confirmed that exposure to acute and chronic heat stress induces oxidative stress in the hearts of chicks. Heat-stress-induced oxidative stress in the heart shows that the transcription factor *NRF2* promotes the activity of the antioxidant enzyme *CAT*, which is evidenced by the same pattern of gene expression under chronic and acute heat stress. The upregulation of *NRF2* and *CAT* expression indicates that these genes are required to detoxify ROS in the heart, thus helping to maintain redox homeostasis and reduce oxidative stress under heat stress.

The findings of this study are in agreement with those of previous studies that examined the effect of chronic and acute heat stress on the oxidation status of different tissues. In one study, the expression of *NRF2* and *CAT* was elevated in mouse testes after exposure to elevated ambient temperatures (42°C) for 2 hours for twelve days. They found that the expression of *NRF2* and *CAT* varied, with the highest expression seen after the first day of heat treatment; and that *NRF2* protected against heat-induced oxidative stress in mouse testes (Li *et al.*, 2013). It has been shown that *NRF2* and *CAT* were upregulated in the liver of broilers at different acute high ambient temperatures; thereby, broilers have certain tolerance to oxidative stress induced by high ambient temperatures (Q. Miao *et al.*, 2020). In addition, high ambient temperatures induce spleen dysplasia in broilers through the activation of the *NRF2* pathway (C. Zhang *et al.*, 2018). Based on the literature, the effects of chronic and acute heat stress-induced oxidative stress in the heart are limited, and our study showed patterns seen in different tissues in the heart.

Our results showed that under chronic heat stress, the transcription factors of inflammation, *NF-kB*, and its proinflammatory cytokine, *LITAF*, are sequentially upregulated. After the 1st week of heat treatment, *NF-kB* was significantly upregualted ($p \leq 0.0001$) in the heat-stressed hearts, but its expression was reduced in the 2nd week, and after the 3rd and 4th weeks, the expression was significantly downregulated ($p \leq 0.001$ and $p \leq 0.0001$, respectively) compared with the first week of heat treatment and the control (Figure 5a). In contrast, the expression of *LITAF* was upregulated during the 2nd and 3rd weeks of heat treatment, compared to the control (Figure 5b). Under acute heat stress, the expression of *NF-kB* was generally higher than that of *LITAF* across the different treatment groups; however, both were downregulated in comparison to the control (Figures 5c and d).

Previous studies have shown that acute and chronic heat stress induce intestinal inflammation in broiler chickens by stimulating the mRNA expression of proinflammatory cytokines (Varasteh *et al.*, 2015). For example, a recent study showed that heat stress increased intestinal inflammatory injury in chickens by increasing the expression of *NF-kB* and *TNF-*α (Tang *et al.*, 2021). Another recent study on the effects of chronic heat stress on liver inflammatory injury showed

that *NF-kB* and *TNF-α* were upregulated in the livers of heat-stressed broilers, compared with the controls (Y.-L. Liu *et al.*, 2022). In our study, we used *LITAF* as a regulator of *TNF-α* gene expression because only few studies have been conducted on the effect of heat stress on *LITAF*. One study showed that broilers exposed to heat stress had higher expression of *LITAF* in the jejunum and ileum than broilers kept in a comfortable environment (Del Vesco *et al.*, 2020).

The expression of *Leptin* during chronic heat stress was very similar to that of *NF-kB*, as it was also significantly upregulated ($p \leq 0.05$) during the 1st week of chronic heat stress (Figure 5a and 6a). Over the following weeks, the expression of both genes declined. Many studies have reviewed the role of *Leptin* as an inflammatory mediator in chronic inflammation and immunity. *Leptin* induces the secretion of proinflammatory and anti-inflammatory cytokines via activation of the *NF-kB* signaling pathway (Procaccini *et al.*, 2009; Pérez-Pérez *et al.*, 2020). Thus, the significant high expression of *Leptin* and *NF-kB* during the 1st week of chronic heat stress might indicate a possible role of *Leptin* in inducing the *NF-kB* inflammatory pathway in hearts with heat stress-induced oxidation. The association between *Leptin* and *NF-kB* has also been proven in other studies; for example, *Leptin* showed anti-apoptotic properties on neutrophils through nuclear *NF-kB* pathway (Sun *et al.*, 2013). A menstrual cycle model study showed an inverse regression of NF-κB p65 activation on *Leptin* concentrations in the initial follicular phase, as well as in the mid-luteal phase (Faustmann *et al.*, 2016). In our study, *Leptin* was mostly expressed in treatment group 1 (45 min) among the acute heat stress groups, but it was still downregulated compared with the control group.

Previous studies have shown that *Leptin* induces oxidative stress by overproducing ROS (Palomba *et al.*, 2015; Schroyen *et al.*, 2012; Blanca *et al.*, 2016; Mahbouli *et al.*, 2017; Shetty *et al.*, 2022). It is possible that the high expression of *Leptin* observed in our study during the 1st week of chronic heat stress led to increased ROS production in the following weeks, corresponding to the peak in the expression levels of *NRF2* and *CAT* during the 2nd week of heat treatment (Figure 4a and b). Therefore, we believe that during chronic heat stress, *Leptin* may play an important regulatory role in inducing oxidative stress, and this induction might be the reason for the upregulation of the expression of antioxidative genes to detoxify ROS in the heart.

CONCLUSIONS

In summary, our study showed that heat stress induces oxidation and inflammation in the hearts of chicks. The induction of inflammatory and anti-oxidation genes was observed more upon exposure to chronic heat stress than upon acute heat stress, which may confirm previous studies that show chronic heat stress being generally more tolerated by animals than acute heat stress. As far as we know, this is the first study to report the expression of *Leptin* in the heart upon exposure to heat stress.

The practical application of this study's findings is significant for understanding the impact of chronic and acute heat stress on the hearts of chickens. By identifying the specific oxidative and inflammatory responses in chick hearts under different heat stress conditions, this research can inform poultry farmers and veterinarians on the risks associated with heat stress and strategies to mitigate them. For example, implementing measures to reduce exposure to prolonged heat stress in poultry farming practices could help prevent cardiac tissue damage and inflammation in chickens. Additionally, the differential gene expression patterns observed in response to chronic and acute heat stress could guide the development of targeted interventions to enhance antioxidant defenses and modulate inflammatory responses in poultry under varying heat stress conditions. Overall, this study contributes to advancing the welfare and health management of chickens in the context of heat stress, highlighting the importance of proactive measures to protect against heat-induced cardiac complications in poultry farming.

ACKNOWLEDGMENTS

The authors would also like to thank Fatma Almousa for participating in sample collection and RNA extraction, and Thecla Gomes for histological staining of the heart samples. The authors would like to acknowledge the assistance of Ms. Sahar Barhoush for the statistical analysis and validation. The authors acknowledge the Animal Care and Breeding Unit and the Micro-technique Unit at the Department of Biological Sciences, The Biotechnology Center (BTC)-Faculty of Science for the use of their facilities, and the general facility project GS 03-01 belonging to the Research Sector Project Unit (RSPU) for the use of liquid nitrogen generator.

Author contributions

NKB was the primary investigator in this project. NKB contributed to the study conception and experimental

design. ZJA and TAA performed experiments and statistical analyses. NKB prepared and wrote a first draft of the manuscript. ZJA and TAA contributed with manuscript revision. All authors have read and approved the submitted version

Funding

Fund for this work was provided by the college of graduate studies of Kuwait University.

Data availability statement

The datasets used and/or analyzed and materials used during the current study are available from the corresponding author upon reasonable request.

Conflicts of interest

No potential conflict of interest was reported by the author(s)

REFERENCES

Abdel-Rahman HG, Alian HA, Mahmoud, MM. Impacts of dietary supplementation with nano-iron and methionine on growth, blood chemistry, liver biomarkers, and tissue histology of heat-stressed broiler chickens. Tropical Animal Health and Production 2022;54(2):126. https://doi.org/10.1007%2Fs11250-022-03130-w

Abdelnour SA, Abd El-Hack ME, Khafaga AF, *et al.* Stress biomarkers and proteomics alteration to thermal stress in ruminants: A review. Journal of thermal Bioloy 2019;79:120-34. https://doi.org/10.1016/j.jtherbio.2018.12.013

Adu-Asiamah P, Zhang Y, Amoah K, *et al.* Evaluation of physiological and molecular responses to acute heat stress in two chicken breeds. Animal 2021;15(2):100106. https://doi.org/https://doi.org/10.1016/j.animal.2020.100106

Aengwanich W, Simaraks S. Pathology of heart, lung, liver and kidney in broilers under chronic heat stress. Pathology 2004;26(3):18. https://www.thaiscience.info/journals/Article/SONG/10462441.pdf

Ahmad R, Yu YH, Hsiao FS, *et al.* Influence of heat stress on poultry growth performance, intestinal inflammation, and immune function and potential mitigation by probiotics. Animals (Basel) 2022;12(17). https://doi.org/10.3390/ani12172297

Akbarian A, Michiels J, Degroote J, *et al.* Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. Journal of animal science and biotechnology 2016;7(1):1-14. https://jasbsci.biomedcentral.com/articles/10.1186/s40104-016-0097-5

Alva R, Mirza M, Baiton A, *et al.* Oxygen toxicity: Cellular mechanisms in normobaric hyperoxia. Cell Biology and Toxicology 2023;39(1):111-43. https://doi.org/10.1007/s10565-022-09773-7

Barnes TL, Burrack RM, Schmidt TB, *et al.* Sustained heat stress elevated corneal and body surface temperatures and altered circulating leukocytes and metabolic indicators in wether lambs supplemented with ractopamine or zilpaterol. Journal of Animal Science 2021;99(9). https://doi.org/10.1093/jas/skab236

Bastaki NK, Albarjas TA, Almoosa FA, *et al.* Chronic heat stress induces the expression of HSP genes in the retina of chickens (Gallus gallus). Fronteirs in Genetics 2023;14: 1085590. https://doi.org/10.3389/fgene.2023.1085590

Bastaki NK, Lobo VR, Gomes T, *et al.* Retinal gene expression of selective genes and histological stages of embryonic and post-hatch chickens (Gallus gallus). Genes 2022;13(11):2048. https://doi.org/10.3390/genes13112048

Birben E, Sahiner UM, Sackesen C, *et al.* Oxidative stress and antioxidant defense. World Allergy Organization Journal 2012;5(1):9-19. https://doi.org/10.1097/wox.0b013e3182439613

Blanca A J, Ruiz-Armenta MV, Zambrano S, *et al.* Leptin induces oxidative stress through activation of nadph oxidase in renal tubular cells: antioxidant effect of L-Carnitine. Journal of Cellular Biochemistry 2016;117(10):2281-8. https://doi.org/10.1002/jcb.25526

Brugaletta G, Teyssier JR, Rochell SJ, *et al.* A review of heat stress in chickens. Part I: Insights into physiology and gut health. Frontiers in Physiology 2022;13:934381. https://doi.org/10.3389%2Ffphys.2022.934381

Bruun JM, Pedersen SB, Kristensen K, *et al.* Effects of pro-inflammatory cytokines and chemokines on leptin production in human adipose tissue in vitro . Molecular and Cellular Endocrinology 2002;190(1-2):91-9. https://doi.org/10.1016/s0303-7207(02)00007-2

Burhans W, Burhans CR, Baumgard L. Invited review: lethal heat stress: the putative pathophysiology of a deadly disorder in dairy cattle . Journal of Dairy Science 2022;105(5):3716-35. https://doi.org/10.3168/jds.2021-21080

Cândido MGL, Tinôco IFF, Albino LFT, *et al.* Effects of heat stress on pullet cloacal and body temperature. Poultry Science 2020;99(5):2469-77. https://doi.org/10.1016/j.psj.2019.11.062

Caruso A, Gelsomino L, Panza S, *et al.* Leptin: a heavyweight player in obesity-related cancers. Biomolecules 2023;13(7):1084. https://doi.org/10.3390/biom13071084

Cervantes M, Cota M, Arce N, *et al.* Effect of heat stress on performance and expression of selected amino acid and glucose transporters, HSP90, leptin and ghrelin in growing pigs. Journal of Thermal Biology 2016;59:69-76. https://doi.org/https://doi.org/10.1016/j.jtherbio.2016.04.014

Daghero H, Doffe F, Varela B, *et al.* Jejunum-derived NF-κB reporter organoids as 3D models for the study of TNF-alpha-induced inflammation. Scientific Reports 2022;12(1):14425. https://doi.org/10.1038/s41598-022-18556-3

Del Vesco AP, Khatlab AdS, Santana TP, *et al.* Heat stress effect on the intestinal epithelial function of broilers fed methionine supplementation. Livestock Science 2020;240:104152. https://doi.org/https://doi.org/10.1016/j.livsci.2020.104152

Donald D, William D. Commercial chicken meat and egg production. Journal of Applied Poultry Research 2002;11(2);224-5. http://dx.doi.org/10.1093/japr/11.2.224

Dridi S, Temim S, Derouet M, *et al.* Acute cold- and chronic heat-exposure upregulate hepatic leptin and muscle uncoupling protein (UCP) gene expression in broiler chickens. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 2008;309(7):381-8. https://doi.org/10.1002/jez.461

Emami NK, Jung U, Voy B, *et al.* Radical response: effects of heat stress-induced oxidative stress on lipid metabolism in the avian liver. Antioxidants (Basel) 2020;10(1). https://doi.org/10.3390/antiox10010035

Faustmann G, Tiran B, Maimari T, *et al.* Circulating leptin and NF-κB activation in peripheral blood mononuclear cells across the menstrual cycle. Biofactors 2016;42(4):376-87. https://doi.org/10.1002/biof.1281

Gao J, Liu M, Guo H, *et al.* ROS induced by streptococcus agalactiae activate inflammatory responses via the TNF-α/NF-κB signaling pathway in golden pompano Trachinotus ovatus (Linnaeus,1758). Antioxidants 2022;11(9):1809. https://doi.org/10.3390%2Fantiox11091809

Ghulam Mohyuddin S, Khan I, Zada A, *et al.* Influence of heat stress on intestinal epithelial barrier function, tight junction protein, and immune and reproductive physiology. BioMed Research International 2022; 8547379:1-11. https://doi.org/10.1155%2F2022%2F8547379

Goel A. Heat stress management in poultry. Journal of Animal Physiology and Animal Nutrition 2021;105(6):1136-45. https://doi.org/10.1111/jpn.13496

Goto H, Nakashima M, Nakashima H, *et al.* Heat acclimation ameliorated heat stress-induced acute kidney injury and prevented changes in kidney macrophages and fibrosis. American Journal of Physiology-Renal Physiology 2022;323(3):F243-F254. https://doi.org/10.1152/ajprenal.00065.2022

Hamissou Maman A, Özlü S, Uçar A, *et al.* Effect of chick body temperature during post-hatch handling on broiler live performance1. Poultry Science 2019;98(1):244-50. https://doi.org/https://doi.org/10.3382/ps/pey395

Hirakawa R, Nurjanah S, Furukawa K, *et al.* Heat stress causes immune abnormalities via massive damage to effect proliferation and differentiation of lymphocytes in broiler chickens. Frontiers in Veterinary Science 2020;7:46. https://doi.org/10.3389/fvets.2020.00046

Horwitz DA, Fahmy TM, Piccirillo CA, *et al.* Rebalancing immune homeostasis to treat autoimmune diseases. Trends in Immunology 2019;40(10):888-908. https://doi.org/10.1016/j.it.2019.08.003

Jacobs P J, Oosthuizen M K, Mitchell C, *et al.* Heat and dehydration induced oxidative damage and antioxidant defenses following incubator heat stress and a simulated heat wave in wild caught four-striped field mice Rhabdomys dilectus. PLoS One 2020;15(11):e0242279. https://doi.org/10.1371/journal.pone.0242279

Koh KK, Park SM, Quon MJ. Leptin and cardiovascular disease: response to therapeutic interventions. Circulation 2008;117(25):3238-49. https://doi.org/10.1161/circulationaha.107.741645

La Cava A, Matarese G. The weight of leptin in immunity. Nature Reviews Immunology 2004; 4(5):371-9. https://doi.org/10.1038/nri1350

Lan, X, Hsieh JCF, Schmidt CJ, *et al.* Liver transcriptome response to hyperthermic stress in three distinct chicken lines. BMC Genomics 2016;17(1):955. https://doi.org/10.1186/s12864-016-3291-0

Lara, LJ, Rostagno MH. Impact of heat stress on poultry production. Animals (Basel) 2013;3(2):356-69. https://doi.org/10.3390/ani3020356

Laursen, TL, Zak RB, Shute RJ, *et al.* Leptin, adiponectin, and ghrelin responses to endurance exercise in different ambient conditions. Temperature 2017;4(2):166-75. https://doi.org/10.1080/23328940.2017.1294235

Li Y, Huang Y, Piao Y, *et al.* Protective effects of nuclear factor erythroid 2-related factor 2 on whole body heat stress-induced oxidative damage in the mouse testis. Reproductive Biology and Endocrinology 2013;11:23. https://doi.org/10.1186/1477-7827-11-23

Lin CH, Tsai CC, Chen TH, *et al.* Oxytocin maintains lung histologicl and functional integrity to confer protection in heat stroke. Scientific Reports 2019; 9(1):18390. https://doi.org/10.1038/s41598-019-54739-1

Liu E, Zhao X, Li C, *et al.* Effects of acute heat stress on liver damage, apoptosis and inflammation of pikeperch (Sander lucioperca). Journal of Thermal Biology 2022;106:103251. https://doi.org/10.1016/j.jtherbio.2022.103251

Liu H-L, Lee Z-X, Chuang T-W, *et al.* Effect of heat stress on oxidative damage and antioxidant defense system in white clover (Trifolium repens L.). Planta 2021;254(5): 103. https://doi.org/10.1007/s00425-021-03751-9

Liu W-C, Pan Z-Y, Zhao Y, *et al.* Effects of heat stress on production performance, redox status, intestinal morphology and barrier-related gene expression, cecal microbiome, and metabolome in indigenous broiler chickens. Frontiers in Physiology 2022;13:761. https://doi.org/10.3389/fphys.2022.890520

Liu Y-L, Ding K-N, Shen X-L, *et al.* Chronic heat stress promotes liver inflammation in broilers via enhancing NF-κB and NLRP3 signaling pathway. BMC Veterinary Research 2022;18(1):289. https://doi.org/10.1186/s12917-022-03388-0

Ma B, Xing T, Li J, *et al.* Chronic heat stress causes liver damage via endoplasmic reticulum stress-induced apoptosis in broilers. Poultry Science 2022;101(10):102063. https://doi.org/https://doi.org/10.1016/j.psj.2022.102063

Mahbouli S, Der Vartanian A, Ortega S, *et al.* Leptin induces ROS via NOX5 in healthy and neoplastic mammary epithelial cells. Oncology Reports 2017;38(5):3254-64. https://doi.org/10.3892/or.2017.6009

Miao Q, Si X, Xie Y, *et al.* Effects of acute heat stress at different ambient temperature on hepatic redox status in broilers. Poultry Science 2020;99(9):4113-22. https://doi.org/10.1016/j.psj.2020.05.019

Min L, Zheng N, Zhao S, *et al.* Long-term heat stress induces the inflammatory response in dairy cows revealed by plasma proteome analysis. Biochemical and Biophysical Research Communications 2016;471(2):296-302. https://doi.org/10.1016/j.bbrc.2016.01.185

Mitchell JP, Carmody RJ. NF-κB and the transcriptional control of inflammation. International Review of Cell and Molecular Biology 2018;335:41-84. https://doi.org/10.1016/bs.ircmb.2017.07.007

Morera P, Basiricò L, Hosoda K, *et al.* Chronic heat stress up-regulates leptin and adiponectin secretion and expression and improves leptin, adiponectin and insulin sensitivity in mice. Journal of Molecular Endocrinology 2012;48(2):129. https://doi.org/10.1530/jme-11-0054

Most MS, Yates DT. Inflammatory mediation of heat stress-induced growth deficits in livestock and its potential role as a target for nutritional interventions: a review. Animals 2021;11(12):3539. https://doi.org/10.3390/ani11123539

Mourmoura E, Papathanasiou I, Trachana, VK, *et al.* Leptin-depended NLRP3 inflammasome activation in osteoarthritic chondrocytes is mediated by ROS. Mechanisms of Ageing and Development 2022;208:111730. https://doi.org/10.1016/j.mad.2022.111730

Murugesan S, Ullengala R, Amirthalingam V. Heat shock protein and thermal stress in chicken. In: Asea AAA, Kaur P, editors. Heat shock proteins in veterinary medicine and sciences. Cham: Springer; 2017. p.79-193. http://dx.doi.org/10.1007/978-3-319-73377-7_6

Nasrolahi A, Hosseini L, Farokhi-Sisakht F, *et al.* Cardioprotective effect of Rosa canina L. methanolic extract on heat shock induced cardiomyocyte injury: An experimental study. Journal of Cardiovascular and Thoracic Research 2020;12(4):286. https://doi.org/10.34172%2Fjcvtr.2020.47

Nawaz A H, Amoah K, Leng, QY, *et al.* Poultry response to heat stress: its physiological, metabolic, and genetic implications on meat production and quality including strategies to improve broiler production in a warming world. Frontiers in Veterinary Science 2021;8:699081. https://doi.org/10.3389/fvets.2021.699081

Ohira T, Higashibata A, Seki M, *et al.* The effects of heat stress on morphological properties and intracellular signaling of denervated and intact soleus muscles in rats. Physiological Reports 2017;5(15). https://doi.org/10.14814/phy2.13350

Palomba L, Silvestri C, Imperatore R, *et al.* Negative regulation of leptin-induced Reactive Oxygen Species (ROS) formation by cannabinoid CB1 receptor activation in hypothalamic neurons. Journal of Biological

Chemistry 2015;290(22):13669-77. https://doi.org/https://doi.org/10.1074/jbc.M115.646885

Park DS, Gu B-H, Park YJ, *et al.* Dynamic changes in blood immune cell composition and function in Holstein and Jersey steers in response to heat stress. Cell Stress and Chaperones 2021;26(4):705-20. https://doi.org/10.1007/s12192-021-01216-2

Pérez-Pérez A, Sánchez-Jiménez F, Vilariño-García T, *et al.* Role of leptin in inflammation and vice versa. International Journal of Molecular Sciences 2020;21(16): 5887. https://doi.org/10.3390%2Fijms21165887

Poetsch MS, Strano A, Guan K. Role of leptin in cardiovascular diseases . Frontiers in Endocrinology 2020;11:354. https://doi.org/10.3389/fendo.2020.00354

Procaccini C, Lourenco EV, Matarese G, *et al.* Leptin signaling: A key pathway in immune responses. Current Signal Transduction Therapy 2009;4(1):22-30. https://doi.org/10.2174/157436209787048711

Quinteiro-Filho WM, Ribeiro A, Ferraz-de-Paula V, *et al.* Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. Poultry Science 2010;89(9):1905-14. https://doi.org/10.3382/ps.2010-00812

Saeed M, Abbas G, Alagawany M, *et al.* Heat stress management in poultry farms: A comprehensive overview. Journal of Thermal Biology 2019;84:414-25. https://doi.org/10.1016/j.jtherbio.2019.07.025

Sánchez-Margalet V, Martín-Romero C, Santos-Alvarez J, *et al.* Role of leptin as an immunomodulator of blood mononuclear cells: mechanisms of action. Clinical and Experimental Immunology 2003;133(1):11-19. https://doi.org/10.1046/j.1365-2249.2003.02190.x

Schroyen B, Guimarães EL, Dollé L, *et al.* Leptin-mediated reactive oxygen species production does not significantly affect primary mouse hepatocyte functions in vitro. European Journal of Gastroenterology & Hepatology 2012;24(12):1370-80. https://doi.org/10.1097/MEG.0b013e328357ce1c

Schulze PC, Kratzsch, J. Leptin as a new diagnostic tool in chronic heart failure. Clinica Chimica Acta 2005;362(1-2):1-11. https://doi.org/10.1016/j.cccn.2005.05.019

Shetty SS, Kumari NS, Hegde P, *et al.* Leptin gene polymorphism Rs7799039; G2548A, metabolic and oxidative stress markers in polycystic ovarian syndrome. Journal of King Saud University-Science 2022;34(6):102222. https://doi.org/10.1016/j.jksus.2022.102222

Sies H, Berndt C, Jones DP. Oxidative stress. The Annual Review of Biochemistry 2017;86:715-48. https://doi.org/10.1146/annurev-biochem-061516-045037

Song JH, Kim KJ, Choi SY, *et al.* Korean ginseng extract ameliorates abnormal immune response through the regulation of inflammatory constituents in Sprague Dawley rat subjected to environmental heat stress 2019. The Journal of Ginseng Research 2019;43(2): 252-60. https://doi.org/10.1016/j.jgr.2018.02.003

Sun Z, Dragon S, Becker A, *et al.* Leptin inhibits neutrophil apoptosis in children via ERK/NF-κB-dependent pathways. PLoS One 2013;8(1):e55249. https://doi.org/10.1371%2Fjournal.pone.0055249

Surai PF, Kochish II, Kidd MT. Redox homeostasis in poultry: regulatory roles of NF-κB. Antioxidants 2021;10(2). https://doi.org/10.3390/antiox10020186

Tang L-P, Li W-H, Liu Y-L, *et al.* Heat stress aggravates intestinal inflammation through TLR4-NF-κB signaling pathway in Ma chickens infected with Escherichia coli O157:H7. Poultry Science 2021;100(5):101030. https://doi.org/https://doi.org/10.1016/j.psj.2021.101030

Tang L-P, Liu Y-L, Zhang J-X, *et al.* Heat stress in broilers of liver injury effects of heat stress on oxidative stress and autophagy in liver of broilers. Poultry Science 2022;101(10):102085. https://doi.org/https://doi.org/10.1016/j.psj.2022.102085

Tang S, Lv Y, Chen H, *et al.* Comparative analysis of αB-crystallin expression in heat-stressed myocardial cells in vivo and in vitro. PLoS One 2014;9(1):e86937. https://doi.org/10.1371%2Fjournal.pone.0086937

Varasteh S, Braber S, Akbari P, *et al.* Differences in susceptibility to heat stress along the chicken intestine and the protective effects of galacto-oligosaccharides. PLoS One 2015;10(9):e0138975. https://doi.org/10.1371/journal.pone.0138975

Vasamsetti SB, Natarajan N, Sadaf S, *et al.* Regulation of cardiovascular health and disease by visceral adipose tissue-derived metabolic hormones. The Journal of Physiology 2013;601(11):2099-120. https://doi.org/10.1113/jp282728

Wei H, Zhang R, Su Y, *et al.* Effects of acute cold stress after long-term cold stimulation on antioxidant status, heat shock proteins, inflammation and immune cytokines in broiler heart. Frontiers in Physiology 2018;9:1589. https://doi.org/10.3389%2Ffphys.2018.01589

Wu X-Y, Wang F-Y, Chen H-X, *et al.* Chronic heat stress induces lung injury in broiler chickens by disrupting the pulmonary blood-air barrier and activating TLRs/NF-κB signaling pathway. Poultry Science 2023;2103066. https://doi.org/10.1016%2Fj.psj.2023.103066

Yamagishi S I, Edelstein D, Du X L, *et al.* Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. The Journal of Biological Chemistry 2001;276(27):25096-100. https://doi.org/10.1074/jbc.M007383200

Yu J, Yin P, Liu F, et al. Effect of heat stress on the porcine small intestine: amorphological and gene expression study. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 2010;156(1):119-28. https://doi.org/https://doi.org/10.1016/j.cbpa.2010.01.008

Zhang C, Chen, K, Zhao X, et al. Protective effects of resveratrol against high ambient temperature-induced spleen dysplasia in broilers through modulating splenic redox status and apoptosis. Journal of the Science of Food and Agriculture 2018;98(14):5409-17. https://doi.org/10.1002/jsfa.9084

Zhang JF, Bai K W, Su WP, et al. Curcumin attenuates heat-stress-induced oxidant damage by simultaneous activation of GSH-related antioxidant enzymes and Nrf2-mediated phase II detoxifying enzyme systems in broiler chickens. Poultry Science 2018;97(4):1209-19. https://doi.org/10.3382/ps/pex408

Zhao F-Q, Zhang Z-W, Wang C, et al. The role of heat shock proteins in inflammatory injury induced by cold stress in chicken heart. Cell Stress and Chaperones 2013;18(6):773-83. https://doi.org/10.1007%2Fs12192-013-0429-8

Disclaimer/Publisher's Note