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# Thermoregulatory, Endocrine, Leukocyte and Hepatic Redox Responses of Wistar Rats (Rattus norvegicus) to Graded Chronic Cyclic Heat Stress

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Abstract

Heat stress (HS) disrupts mammalian homeostasis through interrelated thermoregulatory, endocrine, immune and oxidative pathways, but these pathways are rarely measured together across graded intensities of chronic HS. This study characterized the integrated response of rats to chronic cyclic HS at two intensities. Thirty individually housed male Wistar rats (150 ± 10 g) were randomly assigned to a control group (24 ± 1 °C), mild HS (32 ± 1 °C, 8 h/day) or severe HS (38 ± 1 °C, 8 h/day) for 21 days at 60 ± 5% relative humidity. Relative to the control, final body weight fell by 14.1% and 34.4% under mild and severe HS and feed intake by 15.6% and 42.2%, while the feed conversion ratio increased from 2.65 to 3.04 and 4.33, respectively ($P < 0.001$). During heating under severe HS, rectal temperature rose from 37.2 to 40.8 °C and respiration rate from 85 to 210 breaths/min. One severe-HS rat reached a humane endpoint on day 18; mortality did not differ among groups ($P = 1.000$). In samples collected after 15–17 h at 24 °C, severe HS increased the neutrophil/lymphocyte ratio from 0.34 to 3.20 (9.4-fold) and serum corticosterone from 45 to 160 ng/mL (3.6-fold). Hepatic malondialdehyde rose from 2.5 to 8.5 nmol/mg protein, whereas superoxide dismutase activity decreased from 55 to 18 U/mg protein ($P < 0.001$). Chronic cyclic HS at 38 °C therefore exceeds the compensatory capacity of the rat, producing sustained hypothalamic–pituitary–adrenal activation and hepatic redox imbalance at the cost of growth efficiency.

Keywords

Corticosterone,
Heat Stress,
Neutrophil/Lymphocyte Ratio,
Oxidative Stress,
Thermoregulation.

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## 1. Introduction

Heat stress (HS) is a growing threat to human and animal health as ambient temperatures and the frequency of heat waves rise. Summer temperatures in Iraq regularly exceed 45 °C, and hot temperature extremes have become more frequent over recent decades (Salman et al., 2017). Such heat exposure is associated with reduced feed intake and growth, endocrine and immune dysfunction and, in extreme cases, heat stroke with multi-organ injury (Bouchama and Knochel, 2002; Leon and Bouchama, 2015). Understanding how these responses develop and interact during continued sub-lethal heat exposure is therefore important for understanding both environmental physiology and the welfare of animals kept in hot climates.

The laboratory rat is a widely used model in heat-stress research because its thermal physiology is well documented and its responses can be measured under controlled conditions (Gordon, 1990). Unlike humans, rats lack functional sweat glands on haired skin and therefore have limited means of dissipating heat. The tail is the principal vasomotor heat exchanger: its glabrous skin and arteriovenous anastomoses allow blood flow, and hence dry heat loss, to increase sharply as core temperature rises (Young and Dawson, 1982). As ambient temperature approaches body temperature, the gradient for dry heat loss narrows and rats depend on evaporative cooling, achieved mainly by spreading saliva over the fur and, to a lesser extent, by increasing respiratory frequency (Hainsworth, 1967; Gordon, 1993). Because the thermoneutral zone of the rat lies at approximately 28–30 °C, ambient temperatures of 32–38 °C place a progressively greater load on these mechanisms (Gordon, 1993; Romanovsky, 2018).

A sustained heat load activates the hypothalamic–pituitary–adrenal (HPA) axis. In rats the principal glucocorticoid is corticosterone rather than cortisol, because the adrenal cortex lacks 17α-hydroxylase activity. Corticosterone mobilises energy substrates, stimulates hepatic gluconeogenesis and promotes protein catabolism, and it also redistributes circulating leukocytes, raising neutrophil and lowering lymphocyte numbers (Sapolsky et al., 2000). The neutrophil/lymphocyte (N/L) ratio has therefore been widely used as an integrative index of glucocorticoid-mediated stress in vertebrates (Davis et al., 2008). With repeated exposure, these acute responses may be attenuated through "heat acclimation," which develops over several weeks through cardiovascular, metabolic and cellular adjustments (Horowitz, 2002).

At the cellular level, hyperthermia increases mitochondrial production of reactive oxygen species (ROS). At the same time, blood is diverted from the splanchnic circulation to the skin, exposing the liver and intestine to hypoperfusion and reperfusion injury (Hall et al., 2001). When ROS production exceeds antioxidant capacity, membrane lipids undergo peroxidation, generating products such as malondialdehyde (MDA), and antioxidant enzymes such as superoxide dismutase (SOD) may be inactivated or depleted (Belhadj Slimen et al., 2016).

Most studies in rats have used short-term protocols that induce heat stroke, or have examined a single system at a single exposure level. Consequently, it remains unclear how growth, thermoregulation, glucocorticoid secretion, leukocyte distribution and hepatic redox balance are related during repeated sub-lethal heat exposure, and at what intensity these responses shift from compensation to injury. To address this gap, male Wistar rats were exposed to 32 °C or 38 °C for 8 h daily over 21 days, and all five domains were measured in the same animals. It was hypothesised that each response would be temperature-dependent and that, at 38 °C, compensatory mechanisms would fail, resulting in growth depression greater than that explained by reduced feed intake, sustained HPA activation and hepatic oxidative stress.

## 2. Materials and Methods

### *2.1. Ethical approval*

The experimental protocol was approved before the study began by the Animal Ethics Committee (Approval no: WA-343-2026; 15 January 2026), College of Veterinary Medicine, University of Wasit, Iraq. All procedures complied with the NIH Guide for the Care and Use of Laboratory Animals, and the study is reported in accordance with the ARRIVE 2.0

guidelines (Percie du Sert et al., 2020). Humane endpoints were defined in advance: rats with a rectal temperature above 41.5 °C, prostration for more than 15 min or loss of more than 20% of body weight were removed from the heat and euthanised.

### 2.2. Animals, housing and diet

Thirty male Wistar rats (6–7 weeks old; 150 ± 10 g) were obtained from the animal house of the University of Wasit. Rats were housed individually in standard polycarbonate cages with wood-shaving bedding. Individual housing allowed accurate measurement of feed intake and prevented huddling, which would otherwise act as a confounding behavioural thermoregulatory response. A 12 h light/12 h dark cycle was maintained (lights on at 07:00). Rats had ad libitum access to tap water and a standard pelleted rat diet (18% crude protein, 3000 kcal metabolisable energy/kg, Altromin, Germany) during the study.

### 2.3. Experimental design and heat treatments

After 7 days of acclimatisation at 24 °C, rats were allocated to three treatments (n = 10 per group) by computer-generated randomisation stratified by body weight. Each treatment was applied for 21 days in a separate environmental chamber:

1. Control: temperature maintained continuously at 24 ± 1 °C (standard animal-house temperature).
2. Mild HS: 32 ± 1 °C for 8 h/day (09:00–17:00), with 24 °C for the remaining 16 h.
3. Severe HS: 38 ± 1 °C for 8 h/day (09:00–17:00), with 24 °C for the remaining 16 h.

Relative humidity was maintained at 60 ± 5% in all chambers. Temperature and humidity were logged every 15 min using HOBO MX1101 data loggers (Onset Computer Corporation, USA). Sample size was determined by a priori power analysis based on the variability of hepatic SOD activity reported in chronically heat-stressed Wistar rats (Malyar et al., 2021); ten rats per group provided 80% power to detect a 25% between-group difference at α = 0.05.

### 2.4. Growth performance and mortality

Body weight and feed intake were recorded weekly; spilled feed was collected and subtracted from the amount offered. Body weight gain, cumulative feed intake and feed conversion ratio (FCR; feed intake/body weight gain) were calculated over the 21-day period. Rats were inspected twice daily. The time and circumstances of any death or humane-endpoint euthanasia were recorded, and such animals were excluded from end-point analyses.

### 2.5. Physiological measurements

Rectal temperature and respiration rate were measured weekly in all rats at 14:00, i.e. during the heating period. Rectal temperature was measured with a BAT-12 digital thermometer (Physitemp, USA) using a lubricated probe inserted 2 cm into the rectum. Respiration rate was determined by counting flank movements for 1 min while the rat rested undisturbed in its home cage. Thermal discomfort was scored by an observer blinded to treatment on a 0–3 scale: 0, normal posture and breathing; 1, mild tachypnoea; 2, moderate tachypnoea, saliva spreading and visible ear and tail vasodilation; 3, marked tachypnoea, open-mouth breathing or prostration. Values from the final week of exposure are presented.

### 2.6. Blood and tissue sampling

All surviving rats were sampled between 08:00 and 10:00 on day 21. No heating was applied on the sampling day, so all rats had been held at 24 °C for 15–17 h before sampling. Feed was not withheld. Rats were anaesthetised with isoflurane (5% induction, 2–3% maintenance in oxygen), and blood was collected by cardiac puncture within 2 min of first handling to minimise acute handling effects on corticosterone. Rats were then euthanised with an overdose of ketamine/xylazine, and death was confirmed by bilateral thoracotomy (AVMA, 2020). The liver was removed immediately and rinsed in ice-cold saline, and a portion of the median lobe was snap-frozen and stored at −80 °C.

### 2.7. Haematology

Blood for haematology was collected into $K_2$-EDTA tubes, and total leukocyte counts were obtained using a BC-2800Vet automated haematology analyser (Mindray, China). Differential counts were performed on Wright–Giemsa-stained smears by classifying 100 leukocytes per smear. Absolute neutrophil and lymphocyte counts and the N/L ratio were then calculated for each rat.

### 2.8. Serum biochemistry

The remaining blood was allowed to clot and centrifuged at 3000 × g for 10 min, and serum was stored at −20 °C until analysis. Serum corticosterone was measured with a competitive ELISA validated for rat serum (Corticosterone ELISA Kit, catalog no. 500655, Cayman Chemical, USA), with intra- and inter-assay coefficients of variation of 5.2% and 7.1%, respectively. Glucose, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined with commercial kits (Bio-Diagnostic, Egypt) on a Mindray BS-200 chemistry analyser.

### 2.9. Hepatic oxidative stress markers

Liver tissue was homogenised (10% w/v) in ice-cold 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10,000 × g for 15 min at 4 °C. MDA was quantified in the supernatant by the thiobarbituric acid reaction (Ohkawa et al., 1979), and SOD activity by inhibition of pyrogallol autoxidation (Marklund and Marklund, 1974). Protein was measured by the method of Lowry et al. (1951), and results were normalised to protein content. All laboratory analyses were performed by staff blinded to group allocation.

### 2.10. Statistical analysis

The individual rat was the experimental unit. Before parametric testing, normality of residuals was assessed with the Shapiro–Wilk test and homogeneity of variances with Levene's test. Continuous variables were compared by one-way ANOVA (fixed factor: temperature) followed by Tukey's honestly significant difference test. Ordinal discomfort scores were compared by the Kruskal–Wallis test followed by Dunn's post hoc test with Bonferroni adjustment, and mortality by the Freeman–Halton extension of Fisher's exact test. All analyses were run in SPSS version 26.0 (IBM Corp., USA). Data are presented as mean ± SEM, and $P < 0.05$ was considered significant.

## 3. Results

### 3.1. Growth performance and mortality

Initial body weight did not differ among groups, but final body weight decreased progressively with exposure temperature (Table 1). Relative to the control, final body weight was 14.1% and 34.4% lower under mild and severe HS, body weight gain 26.5% and 64.7% lower, and feed intake 15.6% and 42.2% lower. Because gain was reduced more than intake, FCR increased from 2.65 in the control to 3.04 and 4.33, respectively. No losses occurred in the control or mild HS groups. One rat in the severe HS group was euthanised on day 18 after reaching a predefined humane endpoint; mortality did not differ among groups ($P = 1.000$).

**Table 1:** Growth performance and mortality of male Wistar rats exposed to chronic cyclic heat stress for 21 days.

| Parameter | Control (24 °C) | Mild HS (32 °C) | Severe HS (38 °C) | P-value |
|---|---|---|---|---|
| Initial body weight (g) | 150 ± 5 | 150 ± 5 | 150 ± 5 | NS |
| Final body weight, day 21 (g) | 320 ± 10$^{a}$ | 275 ± 12$^{b}$ | 210 ± 15$^{c}$ | <0.001 |
| Body weight gain (g) | 170 ± 8$^{a}$ | 125 ± 10$^{b}$ | 60 ± 12$^{c}$ | <0.001 |
| Feed intake (g/rat/21 days) | 450 ± 15$^{a}$ | 380 ± 18$^{b}$ | 260 ± 20$^{c}$ | <0.001 |
| FCR (g feed/g gain) | 2.65 ± 0.08$^{a}$ | 3.04 ± 0.12$^{b}$ | 4.33 ± 0.25$^{c}$ | <0.001 |
| Mortality (humane endpoint), n/N (%) | 0/10 (0) | 0/10 (0) | 1/10 (10) | 1.000 |

*Values are mean ± SEM (n = 10, 10 and 9 surviving rats). Mortality was compared with the Freeman–Halton extension of Fisher's exact test. NS, not significant ($P > 0.05$).*

### 3.2. Thermoregulatory responses

All three thermoregulatory indicators increased stepwise with ambient temperature (Table 2). During the heating period, rectal temperature exceeded the control value by 1.3 °C under mild HS and by 3.6 °C under severe HS. Respiration rate increased 1.6-fold and 2.5-fold under mild and severe HS, respectively, and discomfort scores in the severe HS group indicated moderate to marked heat distress.

**Table 2:** Thermoregulatory responses measured at 14:00 during the heating period in the final week of exposure (mean ± SEM; n = 10, 10 and 9).

| Parameter | Control (24 °C) | Mild HS (32 °C) | Severe HS (38 °C) | P-value |
|---|---|---|---|---|
| Rectal temperature (°C) | 37.2 ± 0.1$^{a}$ | 38.5 ± 0.2$^{b}$ | 40.8 ± 0.3$^{c}$ | <0.001 |
| Respiration rate (breaths/min) | 85 ± 5$^{a}$ | 140 ± 10$^{b}$ | 210 ± 15$^{c}$ | <0.001 |
| Thermal discomfort score (0–3) | 0.0 ± 0.0$^{a}$ | 1.3 ± 0.2$^{b}$ | 2.7 ± 0.2$^{c}$ | <0.001 |

*Discomfort scores were analyzed by the Kruskal–Wallis test; superscripts for this row are from Dunn's test.*

### 3.3. Hematological responses

Total leukocyte count increased by 34% under severe HS, and the composition of the leukocyte population shifted markedly (Table 3). Neutrophil counts were 2.1-fold and 4.0-fold higher than control under mild and severe HS, whereas lymphocyte counts were 20% and 57% lower. Consequently, the N/L ratio rose from 0.34 to 0.89 under mild HS and to 3.20 under severe HS.

**Table 3:** Leukocyte counts and neutrophil/lymphocyte ratio on day 21 (mean ± SEM; n = 10, 10 and 9).

| Parameter | Control (24 °C) | Mild HS (32 °C) | Severe HS (38 °C) | P-value |
|---|---|---|---|---|
| Total leukocytes (×10³/μL) | 5.8 ± 0.4$^{a}$ | 6.5 ± 0.5$^{ab}$ | 7.8 ± 0.6$^{b}$ | <0.01 |
| Neutrophils (×10³/μL) | 1.2 ± 0.2$^{a}$ | 2.5 ± 0.3$^{b}$ | 4.8 ± 0.5$^{c}$ | <0.001 |
| Lymphocytes (×10³/μL) | 3.5 ± 0.3$^{a}$ | 2.8 ± 0.2$^{b}$ | 1.5 ± 0.2$^{c}$ | <0.001 |
| N/L ratio | 0.34 ± 0.04$^{a}$ | 0.89 ± 0.08$^{b}$ | 3.20 ± 0.35$^{c}$ | <0.001 |

### 3.4. Serum biochemical responses

Serum corticosterone and glucose increased with each step in temperature (Table 4). In samples collected after 15–17 h at 24 °C, corticosterone in the severe HS group was 3.6 times and glucose 1.9 times the control value. ALT activity increased 1.6-fold under mild HS and 2.7-fold under severe HS, and AST activity 1.3-fold and 1.9-fold, respectively.

**Table 4:** Serum corticosterone, glucose and aminotransferase activities on day 21 (mean ± SEM; n = 10, 10 and 9).

| Parameter | Control (24 °C) | Mild HS (32 °C) | Severe HS (38 °C) | P-value |
|---|---|---|---|---|
| Corticosterone (ng/mL) | 45 ± 5$^{a}$ | 85 ± 8$^{b}$ | 160 ± 15$^{c}$ | <0.001 |
| Glucose (mg/dL) | 95 ± 5$^{a}$ | 125 ± 8$^{b}$ | 180 ± 12$^{c}$ | <0.001 |
| ALT (U/L) | 35 ± 3$^{a}$ | 55 ± 5$^{b}$ | 95 ± 10$^{c}$ | <0.001 |
| AST (U/L) | 85 ± 6$^{a}$ | 110 ± 10$^{b}$ | 165 ± 15$^{c}$ | <0.01 |

### 3.5. Hepatic oxidative stress markers

Heat exposure shifted hepatic redox balance toward oxidation (Table 5). Relative to the control, lipid peroxidation measured as MDA was 1.9 and 3.4 times higher under mild and severe HS, and SOD activity was 36% and 67% lower.

**Table 5:** Hepatic malondialdehyde concentration and superoxide dismutase activity on day 21 (mean ± SEM; n = 10, 10 and 9).

| Parameter | Control (24 °C) | Mild HS (32 °C) | Severe HS (38 °C) | P-value |
|---|---|---|---|---|
| MDA (nmol/mg protein) | 2.5 ± 0.2$^{a}$ | 4.8 ± 0.4$^{b}$ | 8.5 ± 0.7$^{c}$ | <0.001 |
| SOD (U/mg protein) | 55 ± 4$^{a}$ | 35 ± 3$^{b}$ | 18 ± 2$^{c}$ | <0.001 |

*In all tables, means within a row bearing different superscript letters differ significantly (P < 0.05; Tukey's test unless stated otherwise).*

## 4. Discussion

The magnitude of the response depended on exposure temperature, but not linearly: for nearly all variables, the change between 32 and 38 °C exceeded that between 24 and 32 °C. Rats therefore appear able to buffer a moderate heat load, whereas a daily 38 °C cycle exceeds this buffering capacity.

### 4.1. Thermoregulatory capacity and its limits

At 32 °C, ambient temperature was only a few degrees above the upper limit of the rat's thermoneutral zone. The modest 1.3 °C rise in rectal temperature at this level indicates effective but incomplete compensation via tail vasodilation and moderate evaporative cooling (Gordon, 1990). At 38 °C, the gradient for dry heat loss is abolished and ambient temperature may exceed tail skin temperature, so that heat is gained rather than lost. Rats then depend almost entirely on saliva spreading, supplemented by increased respiratory frequency, both of which are energetically costly and limited by body water reserves (Hainsworth, 1967). Mean rectal temperature reached 40.8 °C during heating, close to the 41–42 °C range associated with the onset of heat stroke in rats (Leon and Bouchama, 2015). This narrow safety margin is illustrated by the severe-HS rat that reached the humane endpoint, although mortality did not differ significantly among groups.

Respiration rate rose 2.5-fold under severe HS. Because rats do not pant and respiratory evaporation contributes relatively little to their heat loss, the observed tachypnoea may reflect increased metabolic demand and sympathetic activation as much as evaporative need. Whether this hyperventilation was sufficient to cause respiratory alkalosis cannot be determined, as blood gases were not measured.

### 4.2. Growth depression beyond hypophagia

Reduced feed intake is a common adaptation to heat because it lowers the heat increment of feeding and thus endogenous heat production. In the present study, however, body weight gain under severe HS declined more than feed intake (64.7% vs 42.2%), and FCR increased by 63%. Hypophagia alone therefore cannot account for the growth deficit. Three mechanisms may contribute: the energy cost of evaporative heat loss, corticosterone-driven protein catabolism, and impaired nutrient absorption secondary to heat-induced intestinal hypoperfusion and barrier dysfunction (Hall et al., 2001; Belhadj Slimen et al., 2016). A pair-fed control group would be required to separate the direct effects of heat from those of reduced intake.

### 4.3. Sustained HPA activation and leukocyte redistribution

Corticosterone increased in a graded manner with exposure temperature. Importantly, samples were taken in the morning after 15–17 h at 24 °C, with no heating on the sampling day, and thus in the absence of acute thermal stimulation. The 3.6-fold elevation in the severe HS group therefore reflects sustained rather than acute HPA activation. It also suggests that 21 days of cyclic exposure did not produce complete heat acclimation, which normally attenuates the endocrine response to repeated heat exposure (Horowitz, 2002). The accompanying hyperglycaemia is consistent with glucocorticoid-stimulated hepatic gluconeogenesis supported by mobilisation of amino acids from skeletal muscle, a process that may also contribute to the poor feed efficiency described above (Sapolsky et al., 2000).

Glucocorticoids promote neutrophil demargination and release from the bone marrow and redistribute lymphocytes from the circulation to lymphoid tissues. The resulting neutrophilia and lymphopenia produced a more than nine-fold increase in the N/L ratio under severe HS, closely paralleling the corticosterone response (Davis et al., 2008). The comparatively small rise in total leukocytes (34%) indicates that the change in N/L ratio reflected redistribution rather than a proportional increase in cell numbers. Whether this redistribution reflects reduced immune competence cannot be concluded, because functional tests such as lymphocyte proliferation or antibody responses were not performed.

### 4.4. Hepatic redox imbalance and liver injury

Under severe HS, hepatic MDA increased 3.4-fold and SOD activity fell to one-third of the control value, indicating increased lipid peroxidation together with loss of a key first-line antioxidant enzyme. Two factors are likely involved. First, hyperthermia increases mitochondrial superoxide production. Second, blood diverted from the splanchnic circulation to the skin for heat dissipation exposes the liver to repeated daily cycles of hypoperfusion and reperfusion, which further increase oxidant generation (Hall et al., 2001). Antioxidant enzyme activity often increases after brief heat exposure as an adaptive response. The marked loss of SOD activity after 21 days suggests that this protective response was ultimately overwhelmed, through oxidative inactivation of the enzyme, reduced synthesis, or both.

The parallel rise in serum ALT and AST is consistent with hepatocellular membrane damage secondary to lipid peroxidation. ALT is relatively liver-specific in rats, whereas AST is also present in skeletal and cardiac muscle; the greater relative increase in ALT (2.7-fold vs 1.9-fold) therefore points to a predominantly hepatic source. Elevated aminotransferases are a common feature of heat injury in mammals (Bouchama and Knochel, 2002; Leon and Helwig, 2010); however, histological evidence is needed to establish the extent and nature of the damage.

### 4.5. Integrated interpretation

Taken together, the findings are consistent with a linked physiological sequence. An increasing heat load raises core temperature, recruits evaporative effectors and increases corticosterone secretion, which in turn drives gluconeogenesis, catabolism and leukocyte redistribution, while splanchnic hypoperfusion and hypermetabolism expose the liver to oxidative stress. At 32 °C, every element of this sequence was modest. At 38 °C, the elements converged: core temperature approached the heat-stroke range, growth efficiency declined, corticosterone remained elevated beyond the heating window, and hepatic antioxidant defences were

depleted. The transition between these two exposure levels therefore marks the practical limit of thermoregulatory compensation in the rat under the conditions studied.

### *4.6. Strengths and limitations*

The main strengths of this study are the concurrent measurement of multiple systems in the same animals, the use of two heat intensities, individual housing that permitted accurate intake measurement, and sampling conditions that distinguish sustained from acute endocrine responses. Several limitations should also be noted. Because only one chamber was used per temperature, chamber-specific effects cannot be separated from treatment effects, and the animals within each chamber are not fully independent replicates. The sample size was powered for the primary oxidative outcome only, and the rat removed at the humane endpoint may have been the most heat-sensitive animal, so the effects of severe HS may be underestimated. Water intake, blood gases, inflammatory cytokines and liver histology were not assessed. Finally, the control group was not maintained at strict thermoneutrality, as 24 °C lies below the rat's thermoneutral zone. Future studies should use replicated chambers and pair-fed controls and should include additional antioxidant indices (glutathione peroxidase, catalase and reduced glutathione) and heat shock protein 70, a key mediator of cellular thermotolerance (Kregel, 2002).

## 5. Conclusions

The physiological cost of chronic cyclic heat rose steeply between 32 and 38 °C. Exposure to 32 °C was largely tolerated, although measurable changes in growth, leukocyte profile and hepatic redox status occurred. At 38 °C, core temperature approached the heat-stroke threshold during heating, final body weight fell by 34.4%, feed efficiency declined disproportionately, corticosterone remained elevated after the heating period, the N/L ratio increased more than nine-fold, and hepatic lipid peroxidation increased while SOD activity was depleted. Limiting hyperthermia under severe heat is therefore achieved at the expense of growth, immune balance and hepatic integrity. This model provides a framework for testing antioxidant and other protective interventions, and the transition identified between 32 and 38 °C may help define safe thermal limits for housing laboratory animals in hot climates.

## Declarations

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**Conflict of interest:** The author declares no conflict of interest.

**Author contribution:** Ansam Faisal Yasir conceived and designed the study, carried out the experimental work and laboratory analyses, analysed the data and wrote the manuscript.

**Data availability:** The data supporting the findings of this study are available from the corresponding author on reasonable request.

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