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Short duration heatwaves increase body temperature and alter blood gas balance but may not cause oxidative stress and intestinal structure variations in lambs

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ABSTRACT

This study investigated the impact of short duration heatwaves (HW) on the body temperature, blood gas, intestinal histology, and oxidative stress parameters of second cross lambs. Seventy-two second cross lambs [Poll Dorset × (Border Leicester × Merino)] were selected and exposed to either one, three or five days HW (28–38°C and 40–60 % relative humidity (RH)) or thermoneutral (TN; 18–21°C, 40–55 % RH) conditions in climate-controlled chambers. Lambs exposed to one to five days HW exhibited higher face, eye and ear temperature compared to animals exposed to equal duration under TN conditions. HW also had a significant impact on blood gas parameters which include higher blood pH, and lower CO_2, Ca^{2+}, Na^{+}, $CHCO_3^{-}$ and Cl^{-}. However, HW lambs' histology structure of the ileum and the GSSG: GSH of the ileum and *Longissimus thoracis* (LT) muscle were not influenced ($P > 0.05$) by HW. LT muscle showed higher total antioxidant capacity (TAC) in HW conditions, but the TAC of intestine and blood had no difference between HW and TN groups. These results suggest that short-duration HW (one to five days) had a significant impact on lambs' body temperature and blood parameters, but neither caused oxidative stress nor any changes in their intestinal structure.

1. Introduction

The climate change and global warming will have negative impacts on animal welfare and meat industries. According to recent reports, summer heatwaves (HW) are getting longer, hotter, and more frequent in Australia (Adnan et al., 2022), China (Li et al., 2017) and the United States (Rastogi et al., 2020). Therefore, sheep industries are facing increased challenges of heat stress (HS) from extreme high temperature days in summer (Al-Dawood, 2017). Previous research has shown that summer or high temperatures leads to a higher occurrence of meat quality defects in ruminants (Kadim et al., 2004; Chauhan et al., 2023) and monogastric species (Gregory, 2010) in Oman, United States, and have also been reported to result in milk production dropped by 14 % from low to high THI in Australia (Osei-Amponsah et al., 2020). However, the direct impacts of HS on sheep physiology are poorly understood and may vary depending on the duration and severity of ambient temperature and breed (Zhang et al., 2020).

Our previous studies demonstrated that one to two weeks of cyclic HS (28–40°C, 40–60 % RH) had a negative impact on growth performance and blood gas parameters of second cross lambs (Chauhan et al., 2014a; Joy et al., 2020; Zhang et al., 2021), which supports the consensus that HS leads to a negative impact on sheep growth performance and physiology (Marai et al., 2007). However, the effect of acute (less than one week) HS on sheep physiological parameters, oxidative balance and intestinal integrity of lambs has not been characterized. Intestinal permeability and integrity could be influenced by HS due to the altering tight junction proteins, triggering an inflammatory response, and inducing hypoxia (Pearce and Gabler, 2023; Peng et al., 2023). As short-term heat events are becoming more frequent and severe, it is imperative that producers understand how sheep adapt to acute HS and any associated potential impacts on their physiology. Given that one to five days are the most likely durations of summer HW

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in temperate zones (Adnan et al., 2022), and temperature was about 38℃ (40.62℃ mean max temperature in north Victoria state from 2021 to 2023; Bureau of Meteorology, Australian), this experiment was designed to investigate the impacts of three distinct HW durations—-specifically one day, three days, and five days on the physiology of lambs. The objective of the study is to address the gap in existing research regarding the period of heat stress exposure in sheep and to simulate real-world HW conditions, thereby providing a comprehensive understanding of how different lengths of heat exposure may affect sheep, which is crucial for developing effective strategies to manage livestock welfare under climate change scenarios. In this study, body temperature was measured to assess the heat stress status of lambs, and the blood gas, total antioxidant capacity and intestinal histology were studied to monitor the influence of HW on lambs' body condition within 5 days. We hypothesis that the animal would have a adaption period from acute to chronic heat stress with the physiological parameters change.

2. Materials and methods

The use of animals was approved by the University of Melbourne Faculty of Veterinary and Agricultural Sciences Animal Ethics Committee (AEC ID 1914955.1). Seventy-two second cross female lambs [Poll Dorset × (Merino × Border Leicester)] aged between 9 and 12 months and weighing 49.0 ±7.0 kg were procured from three different breeders across North-East Victoria as reported previously (Zhang et al., 2022). The study was conducted using a randomized 2 ×3 factorial design with six consecutive replications (n = 12). Lambs in each replication were randomly allocated to either HW or thermoneutral (TN) conditions for one, three or five days. Lambs were acclimatized to indoor group feeding (two weeks) followed by one-week individual pen feeding before relocating into metabolism cages (1.0 × 0.5 m with polypropylene slat flooring that has a stable grip preventing sheep from slipping) and exposed to simulated HW or TN conditions in the climatic chambers as reported previously (Zhang et al. 2022). Briefly, after three days of acclimatization in the climatic chambers (18–21°C, 45–55 % RH), lambs were exposed to either TN (18–21°C, 45–55 % RH, n=12 of each replication) or HW [28°C (1600–0800 h) to 38°C (0800–16:00 h), 40–60 % RH, n=12 of each replication] simulating different duration of HW (one, three or five days). The room temperature was increased for the HW treatment to 36–38 °C and RH 40 and 60 % from 0800 h until 1600 h. After 1600 h, the room temperature was maintained at 26–28 °C overnight until the next day at 0800 h. The room temperature and RH were recorded every 30 min using temperature-humidity data loggers (TechBrands, Electus Distribution, Rydalmere, NSW, AU; mounted at 1 m height). The THI was calculated using the following equation: THI= db℃-((0.31–0.31 RH) × (db℃- 14.4)) (Marai et al., 2007). In this experiment, the average THI of HW treatment was 30.1 from 0800 to 1600 h and 23.9 at all other times. Details on the THI data has been published by Zhang et al. (2022). Lambs were individually fed a diet consisting of oaten (25 %) and lucerne (25 %) chaff combined with standard lamb finisher pellets (50 %; 14 % protein, 8 % crude fiber, 2 % added salt, 1 % added urea) formulated as per NRC (2007). Lambs were fed *ad libitum* and offered fresh feed twice daily and, leftover feed discarded at each feeding, and water was always available. Daily feed requirements of the animals were calculated using the equation; feed (kg DM/day) = $W^{0.75}$ × 450/1000/ME, where ME is maintenance energy.

2.1. Body temperature

For body temperature measurements, infrared thermography was used as a non-invasive remote sensing tool to assess changes in heat transfer and blood flow in ruminants by detecting slight variations in body temperature. Previous research from our group has developed a prediction equation to accurately assess sheep rectal temperature based on thermal imaging (Joy et al., 2021). Lambs' forehead temperature was recorded using a thermal camera (FLIR T1050sc, FLIR Systems Inc.; Wilsonville, OR, USA) with < 20 mK sensitivity and a wide temperature range (-40 °C to 2000 °C). The accuracy of the thermal camera was ± 2 °C or ± 2 % of reading at 25 °C for temperatures up to 1200 °C, and the emissivity was 0.985 (Osei-Amponsah et al., 2020; Joy et al., 2021). The camera was kept 0.5 m from the lamb's head while capturing the image. Images were processed using FLIR's ResearchIR Max software (FLIR Systems, 2015, Wilsonville, OR, USA) to record the average face, eye and ear temperature. Thermal images were captured three times per day at 0800 h, 1200 h and 1600 h.

2.2. Blood gas and electrolyte measurement

Blood samples were collected by jugular venipuncture at 1400 h at the end of each treatment period (day one, three or five). Immediately after each blood sample, 1 ml of whole blood was withdrawn and used for blood gas analysis. Blood pH, glucose, lactate, partial pressure of oxygen (pO_2) and carbon dioxide (pCO_2), concentration of hydrogen carbonate ($cHCO_3^-$), Ca^{2+}, Cl^-, K^+ and Na^+ was measured using an Epoc system (Alere Inc., Waltham, MA, USA) version 3.29.0 system software (Sensor Configuration 33.0, Alere Inc., Waltham, MA, USA; (Joy et al., 2020). The remaining blood was collected in heparinized tubes (367874, BD Vacutainer, Franklin Lakes, NJ, USA) and centrifuged at 3000×g for 15 mins at 0–4 ℃. The supernatant (plasma) was stored at −20 ℃ prior to antioxidant analysis.

2.3. Lamb slaughter and muscle and intestinal sample collection

At the end of the treatment period, lambs were slaughtered by a licensed mobile butcher after 12 h fasting (water access was allowed), as reported previously (Zhang et al., 2022). All slaughter procedures were followed per commercial methods, including captive bolt stunning before exsanguination. After slaughter, a 10 g muscle sample of LT and a 2 × 2 cm ileum tissue sample was collected and snap-frozen in liquid nitrogen then stored at −80 °C prior to further analysis for biomarkers of oxidative stress.

2.4. Oxidative stress biomarkers

The oxidative stress biomarkers, total antioxidant capacity (TAC) of plasma, ileum and *Longissimus thoracis et lumborum* (LTL) muscle and concertation of oxidized (GSSG) and reduced glutathione (GSH) of ileum and LTL were measured using commercial assay kits, as per manufacturers' instructions (703002, 709001; Cayman, Ann Arbor, MI, USA). Frozen LT muscle and ileum samples were ground using a tissue grinder with liquid nitrogen (20 s, 30 times/s), then homogenized in Tris-HCL buffer (supplied by the assay kit). After homogenization, samples were centrifuged at 10000 g for 15 min at 4 ℃. The supernatant and plasma were used to measure GSSG and total antioxidant capacity (Liu et al., 2016). The measurement of GSH was conducted using the same supernatant f with the addition of 1 M 2-vinylpridine (Sigma-Aldrich, USA).

2.5. Intestinal histology sampling and assessment

After slaughter, the ileum tissue sample was washed with Tril-HCL (7.0) and then stored in 40 % formalin for one week until cutting and fixation with paraffin. Histology slides were processed by the Melbourne Histology Platform, the University of Melbourne (Nolte et al., 2016) and scanned using Pannoramic SCAN II (3D Histech) with Carl Zeiss Plan-Apochromat 20 ×/NA 0.8, Point Grey Grasshopper 3 CCD monochrome camera and LED-based RGB illumination unit. The histology platform operated independently within this project, ensuring unbiased results by assigning a random ID to each sample for evaluation. Images was stored and processed using Panoramic Scanner (3.0.2 -SCAN150). The histology and pathological changes were assessed by the Phenomics Australia Histopathology and Slide Scanning Service at the University of

Melbourne. Samples were graded at a scale of 0–3 by scoring matrix for the gut (Wlwel, 1990; Nolte et al., 2016), which included crypt architecture (normal, irregular, moderate crypt loss, severe crypt loss), tissue damage (no damage, discrete lesion, mucosal erosion, extensive mucosal damage/ ulceration), inflammatory cell infiltration (occasional infiltration, increasing leukocyte in lamina propria, confluence of leukocytes extending into submucosa, transmural extension of inflammatory infiltrate), and enterocyte hyperplasia (none, mild, moderate, severe).

2.6. Statistical analysis

Statistical analysis was performed using linear mixed model procedures in GenStat 18th edition (2015). For analyzing body temperature, the fixed effects were environmental temperature (HW and TN), duration of temperature treatment (one, three or five days), and time of the day (0800, 1200, 1600 h). For blood gas, histology and oxidative stress parameters, temperature (HW and TN) and treatment duration (one, three or five days) were the fixed factors. Replications and sheep/ carcass ID were used as random terms for both analyses in the model. Results were reported as means and standard errors of difference. Means were separated using LSD and considered to differ significantly when $P \leq 0.05$.

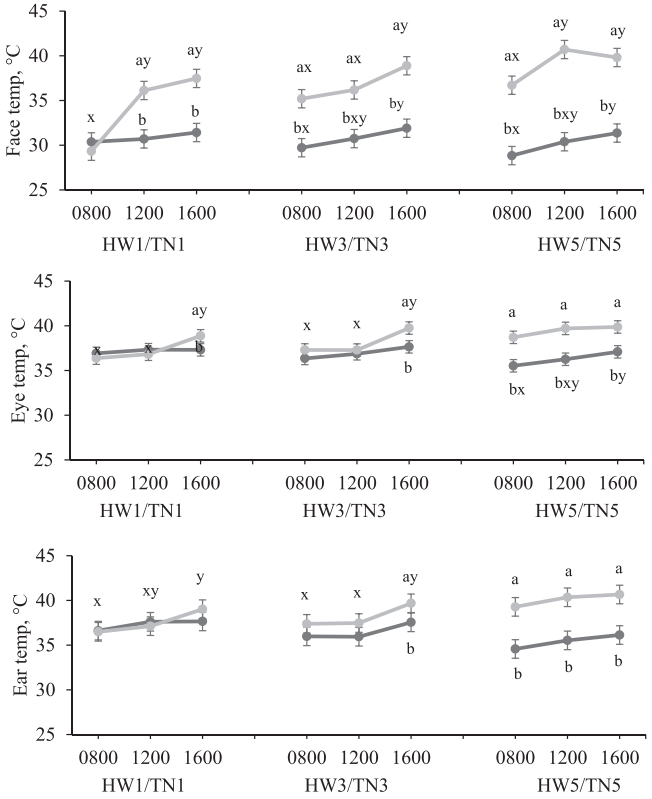

Fig. 1. Lamb body temperature (face, eye and ear average temperature) (0800 h, 1200 h, 1600 h) of the last experiment day (Day five) under different temperatures (thermoneutral, TN vs heatwaves, HW) and heat exposure durations (one, three and five days; n = 12 per group). a-b (compared with different treatments), and x-y (compared with different measurement times) means in a point with different letters are significantly different at a 5 % level of LSD. Values are mean ± SED. [1]Temp. = Temperature. Face, temp. $P < 0.001$; day $P < 0.001$; temp.×day $P < 0.001$. Eye, temp. $P < 0.001$; day $P = 0.011$; temp.×day $P < 0.001$. Ear, temp. $P < 0.001$; day $P = 0.101$; temp.×day $P < 0.001$.

3. Results

3.1. Body temperature

The results of lambs' body temperature were recorded from the face, eye and ear by a thermal camera, as shown in Fig. 1. The average face temperature increased ($P < 0.001$) in HW compared to TN lambs. There was a significant interaction between temperature and experiment days for face, eye and ear temperatures such that eye and ear temperatures of one and three days HW were significantly higher than TN at 1600 h, and the temperature of five days was higher ($P < 0.05$) than TN since 0800 h. The forehead temperatures at three and five days HS treatments at 0800 h were greater ($P < 0.05$) than three and five days TN groups, respectively.

3.2. Blood gas parameters

As shown in Table 1, one, three and five days HW increased ($P < 0.05$), the blood pH and decreased ($P < 0.05$) the pCO_2, Na^+, HCO_3^- and Cl^-. The interaction between temperature and experimental duration was not significant for all blood gas parameters. Among different experiment days, pH, pCO_2 and Cl^- were greater ($P < 0.05$) for three compared to one day HW treatments.

3.3. Intestinal histology

According to the pathology scores (Table 2), one to five days HW had no impact ($P > 0.05$) on ileum histology structure, which reflects the extent of tissue damage, inflammatory cell infiltration, crypt architecture and enterocyte hyperplasia. Heatwave durations increased enterocyte hyperplasia from one to five days but had no significant impact ($P > 0.05$) on other indicators, and the interaction between temperature and experimental days was not significant.

3.4. Oxidative stress biomarkers

As shown in Table 3, one to five days HW significantly increased ($P < 0.05$) TAC of LT muscle but had no impact ($P > 0.05$) on TAC of plasma and ileum, and GSSG: GSH of ileum and muscle. Different HW durations did not significantly alter ($P > 0.05$) oxidative stress parameters except to decrease ($P < 0.05$) TAC in plasma from one to five days. There was no significant interaction between temperature and experiment days among TAC and GSSG: GSH of both ileum and muscle tissues. The TAC of one day HW was greater ($P < 0.05$) than the TN treatment, but this difference was no longer significant as the HW days extended to three and five days.

4. Discussion

This study investigated the effect of short duration HW on sheep physiology and oxidative metabolism with potential implications on intestinal histology. Our results published before (Zhang et al., 2022) and presented here have shown that while short-duration HW cause significant increases in core body temperature and alters blood biochemistry, lambs can cope with short-duration HW and maintain oxidative balance and intestinal integrity.

The Temperature-Humidity Index (THI) is a widely recognized metric for assessing heat stress, calculated by combining ambient temperature and relative humidity data. In this study, chamber room's average THI of HWs reached 30.1 during the daytime and 23.9 at night. This pattern suggests that the lambs were subjected to high-temperature conditions throughout the day, with insufficient nighttime relief to offload the accumulated heat effectively (the THI data has been published before Zhang et al., 2022). As per the THI developed exclusively for sheep, a THI lower than 22.2 is classified as thermoneutral, a THI of 22.2–23.3 is moderate HS, a THI of 23.3–25.6 is classified as severe HS,

Table 1
The effect of temperature (thermoneutral, TN vs heatwave, HW) and heat exposure duration (one vs three vs five days) on blood gas and electrolyte parameters of 2nd cross female lambs (n = 12 per group). a-c Means in the row with the different letters are significantly different at the 5 % level of LSD. TN/HW1 to 5 represents one to five days HW duration.

	1 day		3 days		5 days			*P*-value		
	TN1	HW1	TN3	HW3	TN5	HW5	SED[a]	Temp.[b]	D	T×D[c]
pH	7.48^{ab}	7.57^{c}	7.48^{a}	7.52^{b}	7.46^{a}	7.55^{bc}	0.018	<0.001	0.096	0.20
pCO_2, mmHg	38.7^{c}	27.8^{a}	39.0^{c}	33.0^{b}	39.2^{c}	28.2^{a}	1.96	<0.001	0.12	0.14
Glucose, mmol*L^{-a}	4.08	4.32	4.18	3.96	4.01	4.08	0.183	0.73	0.45	0.23
Lactate, mmol*L^{-a}	0.92	1.10	1.11	2.07	1.14	1.22	0.460	0.14	0.21	0.34
Na, mmol*L^{-a}	147.4^{b}	144.1$_{a}$	147.2^{b}	144.0^{a}	147.5^{b}	143.7^{a}	0.54	<0.001	0.90	0.70
pO_2, mmHg	36.6	38.2	34.3	35.1	34.8	34.8	3.65	0.49	0.68	0.96
Ca^{++}, mmol*L^{-a}	1.34^{ab}	1.30^{a}	1.36$_{b}$	1.32$_{ab}$	1.36^{b}	1.34^{ab}	0.024	0.016	0.22	0.72
$cHCO_3^-$, mmol*L^{-a}	28.9^{c}	25.4^{ab}	28.3^{bc}	26.8^{b}	28.1^{bc}	23.9^{a}	0.92	<0.001	0.046	0.11
Cl^-, mmol*L^{-a}	109.0^{bc}	107.7^{b}	109.3^{c}	106.1^{a}	109.3^{c}	108.3^{bc}	0.700	<0.001	0.10	0.071
K^+, mmol*L^{-a}	4.41	4.36	4.33	4.39	4.38	4.18	0.153	0.48	0.63	0.47

[a] SED = Standard error of means.
[b] Temp. = Temperature.
[c] T×D = Temperature×day

Table 2
The effect of temperature (thermoneutral, TN vs heatwave, HW) and heat exposure duration (one vs three vs five days) on the histology and pathology score of 2nd cross female lambs (n = 12 per group). a-b Means in the row with the different letters are significantly different at 5 % level of LSD. TN/HW1 to 5 represent one to five days HW duration.

	1 day		3 days		5 days			*P*-value		
	TN1	HW1	TN3	HW3	TN5	HW5	SED[a]	*P*-Temp.[b]	*P*-Day	*P*-T×D[c]
Tissue damage	1.67	1.50	1.67	1.33	1.67	1.00	0.301	0.21	0.28	0.16
Inflammatory cell infiltration	2.00	1.67	1.50	1.50	1.50	1.50	0.281	0.50	0.17	0.63
Crypt Architecture	1.00	1.00	1.00	1.00	1.00	0.83	0.096	0.33	0.38	0.38
Enterocyte hyperplasia	1.33^{ab}	1.17^{a}	1.50^{ab}	1.50^{ab}	1.67^{ab}	1.83^{b}	0.271	1.00	0.047	0.69

[a] SED = Standard error of means.
[b] Temp. = Temperature.
[c] T×D = Temperature×day

Table 3
The effect of temperature (thermoneutral, TN vs heatwave, HW) and heat exposure duration (one vs three vs five days) on total antioxidant capacity (TAC) and GSSG: GSH of 2nd cross lambs (n = 12 per group). a-b Means in the row with the different letters are significantly different at the 5 % level of LSD. TN/HW1 to 5 represent one to five days HW duration.

	1 day		3 days		5 days			*P*-value		
	TN1	HW1	TN3	HW3	TN5	HW5	SED[a]	*P*-Temp.[b]	*P*-Day	*P*-T×D[c]
TAC $_{plasma}$, μM/ml	6.99^{a}	6.85^{ab}	6.33^{ab}	6.63^{ab}	6.39^{ab}	6.23^{b}	0.346	0.94	0.047	0.60
TAC $_{ileum}$, μM/g	4.58	4.17	3.95	4.05	4.05	4.18	0.350	0.79	0.27	0.45
GSSG: GSH $_{ileum,}$ μM/g	0.375	0.437	0.467	0.504	0.387	0.385	0.1018	0.60	0.37	0.90
TAC $_{LTL}$, μM/g	0.690^{a}	1.138^{b}	0.728^{ab}	0.935^{ab}	0.731^{ab}	1.009^{ab}	0.2541	0.040	0.91	0.79
GSSG: GSH $_{LTL}$, μM/g	0.312	0.306	0.320	0.311	0.312	0.306	0.0604	0.81	0.97	0.78

[a] SED = Standard error of the of means.
[b] Temp. = Temperature.
[c] T×D = Temperature×day

and when THI exceeds 25.6 it is considered extremely severe HS (Marai et al., 2007).

Body temperature is a common indicator used to assess an animal's thermal balance in HS studies. Like other homeothermic animals, sheep maintain their core body temperature within a narrow range specified for the species under normal conditions (Aggarwal and Upadhyay, 2013), and a rise or decrease of 1 ℃ of core body temperature is enough to cause significant reductions in animal performance (McDowell et al., 1996; Silanikove, 2000; Garner et al., 2017). As reported before (Zhang et al., 2022), the animal body temperature (rectal and skin temperature) was significantly influenced by short-duration exposure to heat (one to five days HWs). The current thermal body temperature data again confirmed that one, three and five days HWs significantly increase the animal body temperature. In this study, the overall increase in body temperature of lambs following exposure to heat stress is also in agreement with our previous longer-term studies, which reported a significant increase in sheep rectal and skin temperatures following exposure to one to two weeks HS (Chauhan et al., 2015; Joy et al., 2020). Compared with one and three days of HW treatment, higher baseline temperatures (face, ear and eye) recorded during the morning of day five of exposure shows that heat accumulation occurs with the repeated exposure as lambs cannot offload heat gained over each exposure day. This typically happens with a HW during natural summer conditions, as hot days are often followed by hot nights (Macías-Cruz et al., 2016). However, this heat accumulation difference among HW treatment did not result in variation in blood gas results in this study.

After one, three and five days of exposure to the simulated HW, lambs showed increased blood pH and decreased pCO_2 compared to TN treatments, demonstrating that lambs exposed to HW (one, three and five days) developed respiratory alkalosis as a result of hyperventilation and the subsequent decrease in pCO_2 due to increased elimination of CO_2 (Srikandakumar et al., 2003b). The higher RR after one to five days

HW treatment in this study (Zhang et al., 2022) also confirms the decrease of pCO_2 in plasma. Along with the reduction in pCO_2, the concentration of HCO_3^- also decreased in this experiment as the excessive CO_2 exhaled is mainly derived from carbonic acid (H_2CO_3), which dissociates to form CO_2 and H_2O (Srikandakumar et al., 2003a). The HCO_3^- is converted to H_2CO_3 by receiving H^+ from the buffer systems, such as hemoglobin, plasma proteins and extracellular fluid phosphates to maintain homeostasis. So the HCO_3^- concentration also decreases with CO_2 concentration in the blood due to the buffering mechanism as HCO_3^- and pCO_2 are kept relatively constant at a ratio of 20:1, which has been demonstrated by previous studies in cows, sheep and poultry (Schneider et al., 1988; Wojtas et al., 2014; Barrett et al., 2019). In a previous sheep HS study, Sivakumar et al. (2010) reported that the pCO_2 and HCO_3^- significantly decreased after 21 days of HS exposure (38–42 °C, 30 % RH) compared to TN (23–27 °C), and a similar decrease was observed in buffaloes (Korde et al., 2007). However, Srikandakumar et al. (2003b) reported an increase in blood HCO_3^- in Merino and Omani sheep from winter (22.2–24.4 ∘C, 60–100 % RH) to summer seasons (35.6–43.0 ℃, 35–95 % RH) which could be because the role of kidney' in acid-base balance. For every molecule of HCO_3^- absorbed, one molecule of H^+ must be secreted by the renal tubules (Bobulescu and Moe, 2006). This mechanism may account for the increased amount of plasma HCO_3^- and the resultant tendency towards alkalosis as seen in heat-stressed animals.

Exposure to one to five days of heatwave (HW) conditions was found to decrease the concentrations of blood electrolytes Na^+, Ca^{2+}, and Cl^-. This study observations are in line with existing studies that report similar reductions in blood electrolytes under HS conditions (Schneider et al., 1988; Ait et al., 1995; Macias-Cruz et al., 2016). This alteration in electrolyte balance is a multifaceted physiological response to HS such as the increase in water consumption, endocrine changes, alterations in renal function, and shifts in electrolyte fluxes across cellular membranes (Sejian et al., 2018). Although the water consumption was not measured in this study, the increase of water intake with similar breed and facility has been reported by Aleena et al., (2020). On the other hand, higher water intake would result the electrolyte losses via increased urinary excretion. For other possible reason, the stress-induced activation of the sympathetic nervous system and subsequent release of catecholamines can lead to transient shifts of electrolytes into cells, further complicating the interpretation of plasma electrolyte concentrations (Borges et al., 2003; Kregel et al., 1996).

Previous study also suggests that heat stress can disrupt the integrity of the gastrointestinal barrier, potentially leading to altered absorption and leakage of electrolytes, thereby contributing to the observed changes in blood electrolyte concentrations (Lambert, 2009). However, the change of intestinal integrity was not observed in this study.

When exposed to high ambient temperatures, sheep try to reduce body heat production and dissipate the extra heat to maintain their core temperature. To achieve this thermal balance, there is increased redistribution of blood supply to the periphery and a reduction in blood flow to visceral organs such as intestines (Kregel et al., 1988). This can potentially result in hypoxia of intestinal tissue, cellular ATP depletion, acidosis and cellular dysfunction, which in severe cases may result in necrosis and shedding of intestinal epithelial cells, and increased intestinal permeability (Hall et al., 2001; Yu et al., 2010). These histological changes have been reported to be associated with oxidative stress and mitogen-activated protein kinase (MAPK) pathway activation (Yu et al., 2012). The MAPK pathway would be activated by oxidative stress, which is a signaling pathway that plays a crucial role in cellular responses to stress, including inflammation, apoptosis, and cell differentiation (Roth, 2017). The MAPK pathway's activation under heat stress condition indicates a cellular attempt to counteract the effects of oxidative stress and restore cellular homeostasis (He et al., 2015). However, persistent activation of this pathway can lead to adverse cellular outcomes, including the promotion of inflammatory processes and further contributing to the cycle of cellular damage, dysfunction, and tissue pathology observed in the intestinal epithelium under heat stress conditions (Yu et al., 2012; Tang et al., 2021).

Despite the severity of the HW protocol employed, in this experiment, we did not observe any significant changes in ileal tissue structure in lambs exposed to one to five days of HW. The histological assessments also did not show any substantial changes in villus structure and mitochondrial swelling indicating that short duration exposure (one to five days) does not cause oxidative damage to intestinal epithelium. Antioxidants assist in the prevention of oxidative damage to tissues. Glutathione (GSH) is an essential scavenger of reactive oxygen species (ROS) and the ratio of its concentration in oxidised form (GSSG) to reduced form (GSH) in tissues can be used as one of the biomarkers of tissue oxidation state and antioxidant levels (Zitka et al., 2012). In this study we did not observe any significant change in GSSG:GSH ratio in intestinal tissue, further confirming that short duration exposure to HS does not affect the oxidative status of intestinal tissues and sheep can maintain the intestinal membrane integrity as was indicated by histological investigations.

While the HW did not affect intestinal tissue, muscle tissue showed more sensitivity to HS. In this study, HW treatment had a significant impact on the TAC of LTL muscle but did not influence the TAC of ileum and plasma. Similar results were observed by Chauhan et al. (2014) that HS (28 – 40 °C) reduced the expression of superoxide dismutase-2 (SOD-2) and HSP70 in LTL muscle but did not change the SOD and glutathione peroxidase (GPx) level of plasma. However, it should be noted that the higher TAC of muscle did not result in the difference in meat quality performance. In this study, we did not observe the influence of HWs on meat colour, water holding capacity and texture in 4 days retail display (Zhang et al., 2022). Results of oxidative stress and meat quality showed that lambs' LTL muscle was able to adapt one to five days HW, as HW did not alter the GSSG:GSH ration between TN and HWs treatment) and change the meat quality. Results from this study suggest that short duration exposure to HS does not affect the oxidative status of sheep as they cope with such conditions by making physiological adaptations. However, long-term HS (more than one month) is known to significantly influence the oxidative status of sheep. For example, Belhadj Slimen et al. (2019) and Rathwa et al. (2017) reported that seasonal HS significantly increased blood SOD and GPx concentrations in sheep. While the results of the impact of chronic HS on sheep oxidative stress are consistent, reports of the impact of short duration HS on the oxidative status of sheep are inconsistent. Kumar et al. (2011) reported that HS (June – July, THI $>$ 79 (equation adapted from Thom (1959)) reduced the blood SOD and increased GPx concentration compared with pre-summer months (March – April, THI = 69.5). On the other hand, our previous study reported that one week of HS exposure (28 – 40 °C) had no impact on plasma SOD and GPx concentrations when compared with TN (18–21 °C) environments (Chauhan et al., 2014a; Chauhan et al., 2014b). Based on the results from the current experiment and previously published data, it is evident that short duration ($\leq$ one week) HS or HW do not affect sheep oxidative status and intestinal integrity, despite the significant perturbations in their physiological parameters which is reflective of the animals' adaptive response to HS.

5. Conclusion

Short-duration HWs ranging from one to five days, elevates the body temperature (measured at the face, eye, and ear) of second cross lambs. This elevation accumulates as the exposure to HW extends from one to five days, leading to a gradual increase in resting body temperature. Furthermore, exposure of lambs to HW also impact blood gas and electrolyte balance, such as higher pH and lower pCO^-, cNa^+, cCa^{2+}, $cCHCO_3^-$ and cCl^-, which is reflective of animals' response to HW condition. However, an absence of any changes in GSSG:GSH ratio of muscle and ileum coupled with no observable differences in intestinal histology, clearly shows that short duration HWs do not affect the tissue oxidative balance. In conclusion, short-duration HWs (from one to five days) do

not cause oxidative stress and intestinal damage in lambs despite the significant perturbations in their physiological parameters and blood gas balance which is reflective of adaptive response of lambs to HS.

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CRediT authorship contribution statement

Minghao Zhang: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Frank R. Dunshea:** Conceptualization, Methodology, Resources, Writing – review & editing. **Aleena Joy:** Investigation, Methodology, Writing – review & editing. **Archana Abhijith:** Investigation, Methodology, Writing – review & editing. **Robyn D. Warner:** Conceptualization, Methodology, Resources, Writing – review & editing. **Krsity DiGiacomo:** Conceptualization, Methodology, Writing – review & editing. **Pragna Prathap:** Investigation, Methodology, Writing – review & editing. **Ting Ma:** Data curation, Investigation. **Surinder S. Chauhan:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Adnan, M.S.G., Dewan, A., Botje, D., Shahid, S., Hassan, Q.K., 2022. Vulnerability of Australia to heatwaves: a systematic review on influencing factors, impacts, and mitigation options. Environ. Res. 213, 113703.
Aggarwal, A., Upadhyay, R., 2013. Heat stress and reproduction. Springer Science & Business Media, New York.
Ait, B., Ait Boulahsen, A., Garlich, J.I.M., Edens, F.W., 1995. Potassium Chloride improves the thermotolerance of chickens exposed to acute heat stress. Poult. Sci. 74, 75–87.
Al-Dawood, A., 2017. Towards heat stress management in small ruminants - a review. Ann. Anim. Sci. 17, 59–88.
Barrett, N.W., Rowland, K., Schmidt, C.J., Lamont, S.J., Rothschild, M.F., Ashwell, C.M., Persia, M.E., 2019. Effects of acute and chronic heat stress on the performance, egg quality, body temperature, and blood gas parameters of laying hens. Poult. Sci. 98, 6684–6692.
Belhadj Slimen, I., Chniter, M., Najar, T., Ghram, A., 2019. Meta-analysis of some physiologic, metabolic and oxidative responses of sheep exposed to environmental heat stress. Livest. Sci. 229, 179–187.
Bobulescu, I.A., Moe, O.W., 2006. Na+/H+ exchangers in renal regulation of acid-base balance. Semin Nephrol. 26, 334–344.
Borges, S.A., Fischer da Silva, A.V., Ariki, J., Hooge, D.M., Cummings, K.R., 2003. Dietary electrolyte balance for broiler chickens exposed to thermoneutral or heat-stress environments. Poult. Sci. 82, 428–435.
Chauhan, S.S., Celi, P., Leury, B.J., Clarke, I.J., Dunshea, F.R., 2014a. Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. J. Anim. Sci. 92, 3364–3374.
Chauhan, S.S., Celi, P., Leury, B.J., Clarke, I.J., Dunshea, F.R., 2014b. Dietary antioxidants at supranutritional doses modulate skeletal muscle heat shock protein and inflammatory gene expression in sheep exposed to heat stress. J. Anim. Sci. 92, 4897–4908.
Chauhan, S.S., Celi, P., Leury, B.J., Dunshea, F.R., 2015. High dietary selenium and vitamin E supplementation ameliorates the impacts of heat load on oxidative status and acid-base balance in sheep1,2. J. Anim. Sci. 93, 3342–3354.
Chauhan, S.S., Zhang, M., Osei-Amponsah, R., Clarke, I., Sejian, V., Warner, R., Dunshea, F.R., 2023. Impact of heat stress on ruminant livestock production and meat quality, and strategies for amelioration. Anim. Front. 13, 60–68.
Garner, J.B., Douglas, M., Williams, S.R.O., Wales, W.J., Marett, L.C., DiGiacomo, K., Leury, B.J., Hayes, B.J., 2017. Responses of dairy cows to short-term heat stress in controlled-climate chambers. Anim. Prod. Sci. 57, 1233–1241.
Gregory, N.G., 2010. How climatic changes could affect meat quality. Food Res. Int. 43, 1866–1873.
Hall, D.M., Buettner, G.R., Oberley, L.W., Xu, L., Matthes, R.D., Gisolfi, C.V., 2001. Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia. Am. J. Physiol. Heart Circ. Physiol. 280, H509–H521.
He, S., Hou, X., Xu, X., Wan, C., Yin, P., Liu, X., Chen, Y., Shu, B., Liu, F., Xu, J., 2015. Quantitative proteomic analysis reveals heat stress-induced injury in rat small intestine via activation of the MAPK and NF-κB signaling pathways. Mol. Biosyst. 11, 826–834.
Joy, A., Dunshea, F., Leury, B., DiGiacomo, K., Clarke, I., Zhang, M., Abhijith, A., Osei Amponsah, R., Chauhan, S.S., 2020. Comparative assessment of thermotolerance in dorper and second-cross (poll dorset/merino × border leicester) lambs. Anim 10, 2441.
Joy, A., Taheri, S., Dunshea, F.R., Leury, B.J., DiGiacomo, K., Amponsah, R.O., Brodie, G., Chauhan, S.S., 2021. Non-invasive measure of heat stress in sheep using machine learning techniques and infrared thermography. Small Rumin. Res, 106592.
Kadim, I.T., Mahgoub, O., Al-Ajmi, D.S., Al-Maqbaly, R.S., Al-Mugheiry, S.M., Bartolome, D.Y., 2004. The influence of season on quality characteristics of hot-boned beef m. longissimus thoracis. Meat Sci. 66, 831–836.
Korde, J.P., Singh, G., Varshney, V.P., Shukla, D.C., 2007. Effects of long-term heat exposure on adaptive mechanism of blood acid-base in buffalo calves. Asian-Austral J. Anim. Sci. 20, 742–747.
Kregel, K.C., Wall, P.T., Gisolfi, C.V., 1988. Peripheral vascular responses to hyperthermia in the rat. J. Appl. Physiol. 64, 2582–2588.
Kumar, M., Jindal, R., Nayyar, S., 2011. Influence of heat stress on antioxidant status in Beetal goats. Indian J. Anim. Res 17, 178–181.
Li, Y., Ding, Y.H., Li, W.J., 2017. Observed trends in various aspects of compound heat waves across china from 1961 to 2015. J. Meteor. Res-Prc 31, 455–467.
Liu, F., Cottrell, J.J., Furness, J.B., Rivera, L.R., Kelly, F.W., Wijesiriwardana, U., Pustovit, R.V., Fothergill, L.J., Bravo, D.M., Celi, P., Leury, B.J., Gabler, N.K., Dunshea, F.R., 2016. Selenium and vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat-stressed pigs. Exp. Physiol. 101, 801–810.
Macias-Cruz, U., Lopez-Baca, M.A., Vicente, R., Mejia, A., Alvarez, F.D., Correa-Calderon, A., Avendano-Reyes, L., 2016. Effects of seasonal ambient heat stress (spring vs. summer) on physiological and metabolic variables in hair sheep located in an arid region. Int. J. Biometeorol. 60 (8), 1279–1286.
Marai, I.F.M., El-Darawany, A.A., Fadiel, A., Abdel-Hafez, M.A.M., 2007. Physiological traits as affected by heat stress in sheep—a review. Small Rumin. Res. 71, 1–12.
McDowell, L.R., Williams, S.N., Hidiroglou, N., Njeru, C.A., Hill, G.M., Ochoa, L., Wilkinson, N.S., 1996. Vitamin E supplementation for the ruminant. Anim. Feed Sci. Technol. 60, 273–296.
Nolte, T., Brander-Weber, P., Dangler, C., Deschl, U., Elwell, M.R., Greaves, P., Hailey, R., Leach, M.W., Pandiri, A.R., Rogers, A., Shackelford, C.C., Spencer, A., Tanaka, T., Ward, J.M., 2016. Nonproliferative and proliferative lesions ofthe gastrointestinal tract, pancreas andsalivary glands of the rat and mouse. J. Toxicol. Pathol. 29, 1S–125S.
Osei-Amponsah, R., Dunshea, F.R., Leury, B.J., Cheng, L., Cullen, B., Joy, A., Abhijith, A., Zhang, M.H., Chauhan, S.S., 2020. Heat stress impacts on lactating cows grazing australian summer pastures on an automatic robotic dairy. Anim. (Basel) 10.
Pearce, S.C., Gabler, N.K., 2023. Hypoxia exacerbates heat stress effects on the porcine intestinal epithelium in vitro. Front. Anim. Sci. 4.
Peng, M., Yi, W., Murong, M., Peng, N., Tong, H., Jiang, M., Jin, D., Peng, S., Liang, W., Quan, J., Li, M., Shi, L., Xiao, G., 2023. Akkermansia muciniphila improves heat stress-impaired intestinal barrier function by modulating HSP27 in Caco-2 cells. Micro Pathog. 177, 106028.
Rastogi, Rastogi, D., Lehner, F., Ashfaq, M., 2020. Revisiting recent U.S. heat waves in a warmer and more humid climate. Geophys. Res. Lett. 47.
Rathwa, S.D., Vasava, A.A., Pathan, M.M., Madhira, S.P., Patel, Y.G., Pande, A.M., 2017. Effect of season on physiological, biochemical, hormonal, and oxidative stress parameters of indigenous sheep. Vet. World 10, 650–654.
Roth, Z., 2017. Effect of heat stress on reproduction in dairy cows: insights into the cellular and molecular responses of the oocyte. Annu Rev. Anim. Biosci. 5, 151–170.
Schneider, P.L., Beede, D.K., Wilcox, C.J., 1988. Nycterohemeral patterns of acid-base status, mineral concentrations and digestive function of lactating cows in natural or chamber heat-stress environments. J. Anim. Sci. 66, 112–125.
Silanikove, N., 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. Livest. Prod. Sci. 67, 1–18.
Sivakumar, A.V.N., Singh, G., Varshney, V.P., 2010. Antioxidants supplementation on acid base balance during heat stress in goats. Asian Austral J. Anim. 23, 1462–1468.
Srikandakumar, A., Johnson, E.H., Mahgoub, O., 2003b. Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. Small Rumin. Res 49, 193–198.
Srikandakumar, Srikandakumar, A., Johnson, E.H., Mahgoub, O.A., 2003a. Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. Small Rumin. Res. 49, 193–198.

Tang, M., Yuan, D., Liao, P., 2021. Berberine improves intestinal barrier function and reduces inflammation, immunosuppression, and oxidative stress by regulating the NF-κB/MAPK signaling pathway in deoxynivalenol-challenged piglets. Environ. Pollut. 289, 117865.
Thom, E.C., 1959. The discomfort index. Weatherwise 12, 57–61.
Wlwel, M.R., 1990. Small and large intestine. Pathol. Fisch. Rat., Ref. Atlas 43–61.
Wojtas, K., Cwynar, P., Kolacz, R., 2014. Effect of thermal stress on physiological and blood parameters in merino sheep. B Vet. I Pulawy 58, 283–288.
Yu, J., Liu, F., Yin, P., Zhao, H., Luan, W., Hou, X., Zhong, Y., Jia, D., Zan, J., Ma, W., Shu, B., Xu, J., 2012. Involvement of oxidative stress and mitogen-activated protein kinase signaling pathways in heat stress-induced injury in the rat small intestine. Stress (Amst., Neth.) 16, 99–113.
Yu, J., Yin, P., Liu, F., Cheng, G., Guo, K., Lu, A., Zhu, X., Luan, W., Xu, J., 2010. Effect of heat stress on the porcine small intestine: a morphological and gene expression study. Comp. Biochem Physiol. A Mol. Integr. Physiol. 156, 119–128.
Zhang, M., Dunshea, F.R., Warner, R.D., DiGiacomo, K., Osei-Amponsah, R., Chauhan, S. S., 2020. Impacts of heat stress on meat quality and strategies for amelioration: a review. Int J. Biometeorol. 64, 1613–1628.
Zhang, M., Warner, R.D., Dunshea, F.R., DiGiacomo, K., Joy, A., Abhijith, A., Prathap, P., Ma, T., Chauhan, S.S., 2022. Impact of heatwaves on the physiology and retail meat quality of lambs. Foods 11, 414.
Zitka, O., Skalickova, S., Gumulec, J., Masarik, M., Adam, V., Hubalek, J., Trnkova, L., Kruseova, J., Eckschlager, T., Kizek, R., 2012. Redox status expressed as GSH:GSSG ratio as a marker for oxidative stress in paediatric tumour patients. Oncol. Lett. 4, 1247–1253.