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RESEARCH

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Microscopic alterations and adaptation in the lungs of the Japanese quail exposed to acute or chronic heat stress

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Abstract

Background Global warming causes heat stress, a significant bioclimatic factor affecting poultry welfare. The effects of heat stress on the morphology of the Japanese quail lungs were investigated in this study. A total of 16 adult Japanese quail were randomly distributed into four groups: a control group (CT), acute heat stress (AH), chronic heat stress with 7 days (CH1) and chronic heat stress with 28 days exposure (CH2). The CT group were maintained at 25 °C temperature, the AH group were exposed to 38 °C temperature for 24 h, and the CH groups were exposed to 35 °C for seven and 28 days. At the end of exposure, the birds were euthanised, and lung tissues were collected and processed for microscopy. Tissue sections were stained using H&E stain, Gomori's stain and immunofluorescence labelling.

Results This study showed no significant difference in body weight, cloacal temperature, respiratory rate and lung parameters in heat-stressed groups compared with the control group. However, microscopic analyses revealed blood congestion, leakage of blood into the airway, inflammatory response and tissue breakage in the heat-stressed groups.

Conclusions Heat stress harmed the lungs of the Japanese quail based on duration and intensity. The negative impact could cause instant mortality, but if the quail survives the initial impact, it can adapt to the heat stress with long-term consequences on its performance.

Keywords Heat stress, Japanese quail, Avian lungs, Morphology, Global warming, Food security

Background

Extreme heat conditions affect poultry welfare, especially in sub-Saharan countries (Nyoni et al., 2019), causing substantial economic loss (Bohler et al., 2021). As a result, several strategies have been investigated to mitigate heat stress in poultry farming (Olgun et al., 2021). The Japanese quail (*Coturnix japonica*) has the potential to provide additional income and protein food security in sub-Saharan Africa due to its small size, easy husbandry and short life cycle (Baer et al., 2015). Furthermore, biomedical and poultry researchers use Japanese quail as a model for human and poultry research (Mizutani, 2003). For example, the Japanese quail is the best model for embryology and hereditary diseases experiments, because of its short generational interval, compared with mice and chicken (Baer et al., 2015). However, heat stress threatens the Japanese quails' welfare due to continued global warming (McKechnie & Wolf, 2010).

Heat stress increases cellular energy demand leading to excessive production of reactive oxygen species

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(Akbarian et al., 2016). Consequently, there is a depletion of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase, leading to oxidative stress and cell damage (Akbarian et al., 2016). Heat-stressed Japanese quail showed low serum antioxidant enzymes and elevated serum and tissue markers of lipid peroxidation called malondialdehyde (Sahin et al., 2017). Reactive oxygen species interact with the lipid membrane, causing peroxidation and compromising the cell wall integrity, leading to water influx and cell swelling (Swanson et al., 2010). In addition, the interaction of the reactive oxygen species with cell proteins causes DNA and RNA damage resulting in cell death (Swanson et al., 2010).

Widespread inflammation ensued from this uncontrolled cell death, leading to tissue damage (D'Arcy, 2019). For example, in the Japanese quail testis, heat stress reduced sperm count, anti-apoptotic marker Bcl-2 and androgen receptors; however, it increased apoptotic markers Bax and lipid peroxidation (Turk et al., 2015). Similarly, Mohamed et al. (2015) reported severe liver congestion, hepatocyte necrosis and fat accumulation in Japanese quail exposed to heat stress. In addition, renal tubular necrosis, neuron degeneration in the brain and lung congestion were caused by heat stress (Mohamed et al., 2015).

The lungs receive a large volume of heat directly during the evaporative cooling to combat heat stress. However, no study elucidated the effect of heat stress on the lungs of the Japanese quail. Hence, this study investigated the microscopic effects of acute and chronic heat stress on the morphology of the Japanese quail lungs.

Methods

Study animals and experimental design

A total of sixteen ($n=16$) adult Japanese quail (5–6 weeks old) were acquired from a commercial farm through the Wits Research Animal Facility. After 1 week of acclimatisation, the birds were randomly assigned into four groups—four (4) birds in each group—based on their initial body weight: a control group (CT), an acute heat stress group (AH), a chronic heat stress group with seven (7) days of exposure (CH1) and a chronic heat stress group with twenty-eight (28) days of exposure (CH2). The CT group were maintained at 25 °C thermoneutral temperature, while AH group was exposed to 38 °C temperature for twenty-four (24) hours, and the CH1 and CH2 groups were exposed to 35 °C for seven (7) and twenty-eight (28) days, respectively. Food and water were provided ad libitum.

The birds were housed in a perspex cage of dimensions: 1 m × 1 m × 0.5 m in size (L × W × H), divided into four compartments with a mesh wire to allow the birds to see each other. A carefully centred infrared bulb (heat source) was connected to a programmable thermocouple that switched off the heat once the desired temperature level is reached. In addition, a thermostat is attached to the thermocouple. This was used to detect a drop in temperature, which then turned on the heat source to reach the desired temperature again. Cage humidity was maintained with the aid of wet nestlets. A data logger was placed in the chamber to monitor temperature and humidity levels. The top of the cage was covered for about a quarter with a wood flank to allow access to the birds for feeding and routine procedures, and the remainder was thatched with straw to allow for ventilation while minimising thermal drift. These measures ensure all the bird chambers get equal heat distribution at the centre and can use the space towards the periphery to escape the heat. Respiratory rate, body temperature and body weight were monitored daily throughout the experiment duration.

At the end of the experiment, the birds were euthanised with an intravenous (wing vein) injection of sodium pentobarbitone at 120 mg/kg body weight. The birds were placed in a supine position, and the trachea was accessed through an incision at the level of the syrinx. Neutral buffered formalin fixative was instilled through the trachea while gently massaging the bird's abdomen to ensure the air within the lungs was replaced with the fixative and the trachea was ligated. The tissue is allowed to fix for two minutes and then excised and immersed into the same fixative. The lung weight was measured using a digital scale after fixation. In addition, lung volume was measured using Scherle's technique based on the Archimedes principle (Scherle, 1970). Lung size was also estimated using a vernier calliper as the product of width, length and thickness.

Tissue sampling

The fixed lungs were sectioned along the costal sulci. Then, two blocks of cranial and middle tissue from each bird ($n=4$) were selected for processing using an automatic tissue processor Citadel 1000 (Shandon, England). Subsequently, 5-µm serial sections were cut using the rotary microtome Leica RM2125 RTS (Leica Biosystems, Germany). Some sections were collected using silane-coated slides and stained using Mayer's H&E (Bancroft & Layton, 2019), the new pentachrome (Doello, 2014) and Gomori's one-step trichrome (Gomori, 1950) stains and coverslipped with entellan mounting media (Merck, Germany). Some sections were picked up using *Histobond*+slides (Marienfeld, Germany) for immunofluorescence labelling.

Immunofluorescence triple labelling

The sections selected for the immunofluorescence triple labelling were dewaxed and rehydrated, and antigen retrieval was done at 50 °C in citrate buffer (pH 6.0). After twelve (12) hours, the sections were removed from the water bath, allowed to cool and then washed in PBS. After washing, the sections were incubated with 10% normal goat serum for 1 h, followed by the anti-α smooth muscle actin (α-SMA, green) antibody (ab150301, Abcam, UK) (1:500) for twelve (12) hours at 4 °C. Again the sections were washed in PBS, and while working in the dark, the sections were incubated with goat anti-rabbit Alexa fluor 488 (Life Technologies, NL) (1:500) for one (1) hour. Subsequently, the sections were incubated with anti-collagen 1 (Col1, red) antibody (ab88147, Abcam, UK) (1:300) overnight at 4 °C. Finally, on the third day, the sections were incubated with goat anti-mouse Alexa fluor 594 (1:500) (Life Technologies, NL) for 1 h, followed by 4′,6-diamidino-2-phenylindole (DAPI, blue) (Life Technologies, NL) (1:8000) for five minutes. The sections were coverslipped with fluoromount (Sigma Aldrich, USA) and kept at 4 °C until viewing.

Microscopic imaging

Micrographs of tissue sections were taken on an Olympus BX 63 optical fluorescence microscope (Olympus, Japan) fitted with an Olympus DP80 digital camera (Olympus, Japan) using the Olyvia CellSens professional imaging software (Olympus, Japan).

Statistical analyses

All data were sorted using Microsoft excel 365 (Microsoft, USA), and statistical analyses were conducted using GraphPad Prism 8 (GraphPad Software, USA). The results of body weight, cloacal temperature, respiratory rate and lung parameters were analysed using a one-way ANOVA (95% CI) after a normality test using the Shapiro–Wilk test. The micrograph data were mainly qualitative; no statistical test was required.

Results

At the termination of the experiment, no mortality was recorded. The mean of the cloacal temperature, respiratory rate and body weight did not statistically differ between the groups (Table 1). Body weight in the AH group was reduced while CH1 and CH2 gained compared with the control. The respiratory rate increased in AH and CH1 groups while it remained normal in CH2 compared with the control. The mean lung weight significantly differed between the groups using ANOVA, while the mean lung volume and size did not (Table 2). However, Dunnett's mean pairwise comparison of the lung weight revealed that only AH significantly differed from the control ($P=0.0129$). In addition, all the parameters indicate a similar decrease in the AH and CH1 and an increase in the CH2 group compared to the control.

In the representative H&E-stained micrograph (Fig. 1) of the Japanese quail lungs, the parabronchial wall

Table 1 Vital signs of the Japanese quail recorded (body weight, body temperature and respiratory rate) during the experiment in control and heat-exposed groups

	Body weight (g)		Body temperature °C		Respiratory rate (breath/min)	
	Initial	Final	Initial	Final	Initial	Final
CT	232.63 ± 13.55	233.5 ± 20.29	42.03 ± 0.3	42.3 ± 0.2	106 ± 18	98 ± 30
AH	219.38 ± 8.63	214.13 ± 7	42.22 ± 0.6	42.4 ± 0.4	97 ± 4	103 ± 11
CH1	237.5 ± 20.1	246.88 ± 13.17	42 ± 0.6	42.5 ± 0.5	100 ± 18	106 ± 25
CH2	201.13 ± 20.68	223.63 ± 37.34	42.3 ± 0.5	42.4 ± 0.1	99 ± 27	92 ± 16
Two-way ANOVA	$P=0.0836$ F = 2.825		$P=0.8073$ F = 0.325		$P=0.6298$ $F=0.5958$	

The results are shown as mean ± SD. No statistical difference was detected.

Table 2 Japanese quail lung parameters measured after the termination of the experimental heat exposure experiment in control and heat-exposed groups

	CT	AH	CH1	CH2	One-way ANOVA
Lung weight (g)	4.56 ± 0.37	3.56 ± 0.53*	4.24 ± 0.29	4.33 ± 0.38	$P=0.0274$ $F=4.339$
Lung volume (cm^3)	2.58 ± 0.18	2.42 ± 0.61	2.48 ± 0.29	2.55 ± 0.14	$P=0.9203$ $F=0.1613$
Lung size (mm^3)	3818 ± 320.4	3182 ± 332.6	3404 ± 544.7	3596 ± 222.1	$P=0.1530$ $F=2.105$

The results are shown as mean ± SD. * indicates a significant difference compared with the control group.

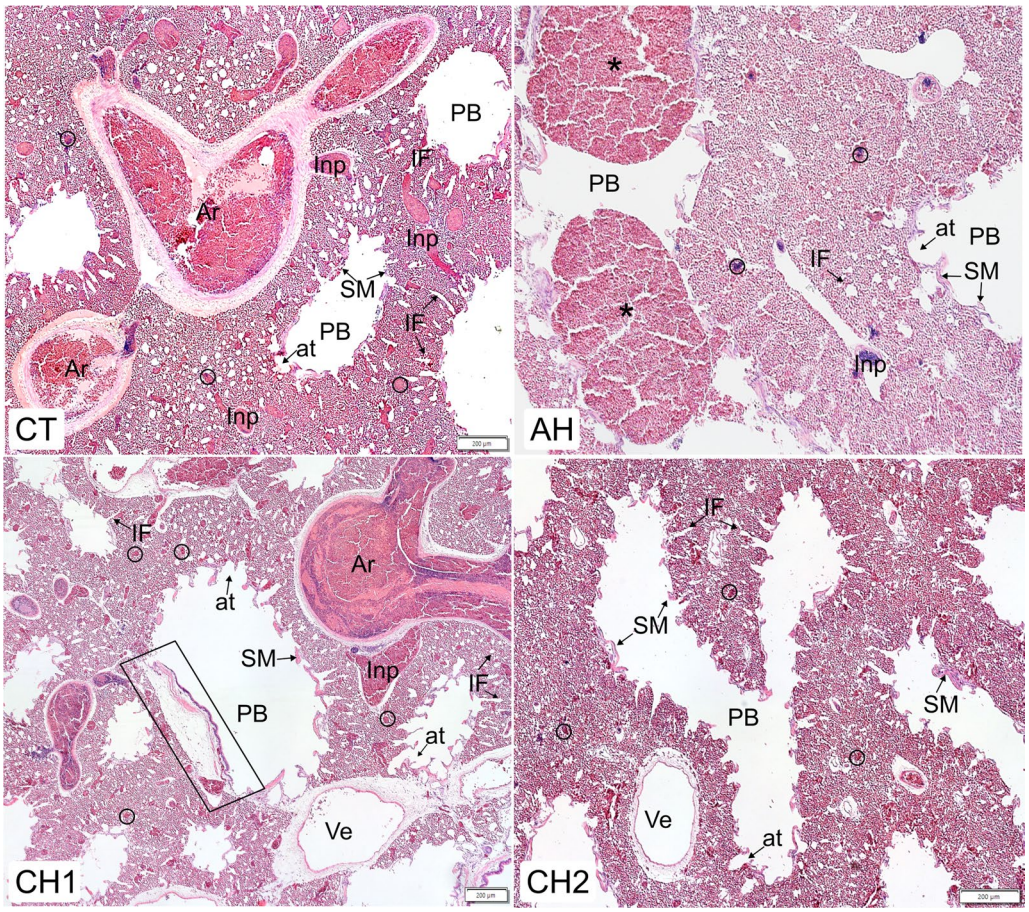

Fig. 1 Representative micrographs of the H&E-stained Japanese quail lungs of the control and heat stress groups. * indicates coagulated blood within the parabronchial lumen (PB). Ar—major artery branch; Ve—major venous branch; SM—smooth muscle; at—atria; if—infundibulae; Inp—inter-parabronchial blood vessel; black circles—intra-parabronchial blood vessel showing increased basophilic-stained cell in the AH group compared to control. The rectangle indicates an area of intersection between secondary bronchi and parabranchi. Note that the blood capillaries, air capillaries and the blood-gas barrier are not visible at this magnification. Scale bar = 200 µm, Mag = 100×

forms the exchange tissue mantle. Embedded within the exchange tissue mantle are the inter-parabronchial and the intra-parabronchial blood vessels which eventually branched into the blood capillaries. The exchange tissue mantle separates the parabronchial lumen from the major blood vessels. Within the wall of the parabronchi, the atria—guarded by interatrial septa—are the primary entrance into the exchange tissue mantle. The infundibulae arise from the atria, leading deeper into the exchange tissue mantle, and branch into the air capillaries, intermingling with the blood capillaries at the blood-gas barrier for gas exchange. In the control group, the blood within the vessels contains more eosinophilic-stained cells indicating erythrocytes. In the AH group, blood contains more basophilic-stained cells indicating an increase in white blood cells, but also note the presence of a significant amount of coagulated blood within the parabronchial airway. In addition, tissue breaks within the parenchyma, which might be from processing or the direct effect of heat was noticed, especially in the Gomori's trichrome. In the CH1 and CH2 groups, the blood within arterioles showed more basophilic-stained cells than the control.

In the new pentachrome-stained Japanese quail lungs (Fig. 2), there are ruptured blood vessels and increased deposition of collagen fibres in the CH1 group. The arrangement of the collagen fibres in the AH and CH1 was irregular compared with the nicely arranged collagen fibres in the CT and CH2, especially at the base of the interatrial septa.

In Gomori's one-step trichrome stain (Figs. 3, 4), the control group showed uniform blood distribution within the exchange tissue mantle. There are a few erythrocytes within the parabronchial lumen, and the smooth muscle appears normal. The blood contains mostly eosinophilic-stained cells indicating a predominance of erythrocytes.

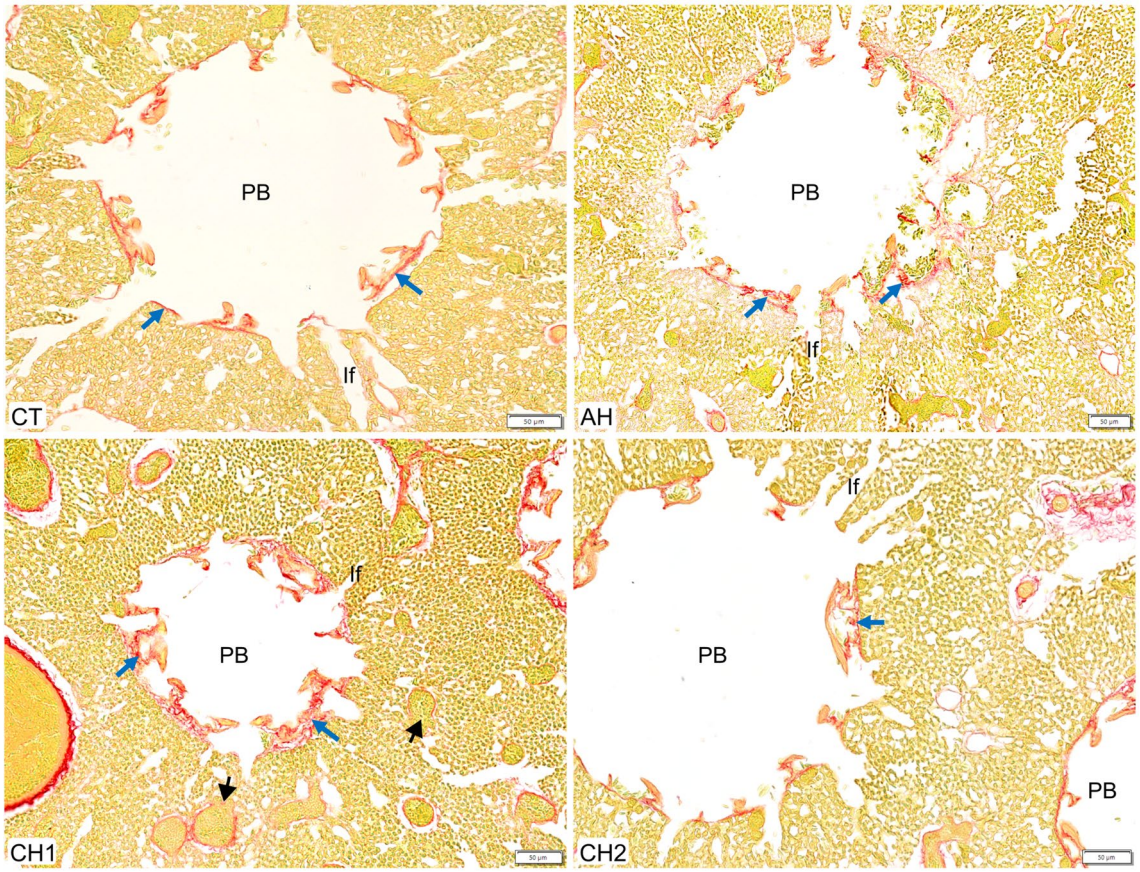

Fig. 2 Representative micrographs of the new pentachrome-stained Japanese quail lungs of control and heat-stressed groups. Note the ruptured blood vessel in the CH1 group indicated with the black arrow. The other blood vessel shows blobs but has not ruptured yet. Blue arrows indicate the unwinding of the collagen fibres in heat-exposed groups compared to control. In addition, there are erythrocytes within the airway in the control group. Normally some erythrocytes will leak into the airway during respiratory cycles which will be removed by surfactants. The collagen fibres show an irregular pattern in the AH and CH1 groups. PB—parabronchial lumen; If—infundibulae. Scale bar = 50 µm, Mag = 400×

In the AH and CH1 groups, most parenchyma regions show blood congestion. The parabronchial lumen has an excessive amount of erythrocytes, and the smooth muscle appears darker than the control group. In addition, the blood shows more basophilic-stained cells indicating the predominance of white blood cells. In the CH2 group, the parenchyma shows uniform blood distribution similar to the control. However, there is a significant amount of erythrocytes within the lumen, and the blood contains basophilic-stained cells. Interestingly, the smooth muscle profile appears normal compared with the control.

In the immunofluorescence representative micrographs (Figs. 5, 6, 7), the α-SMA intensity of the blood vessels is similar in the experimental groups—AH, CH1 and CH1—compared with the control. However, the α-SMA of the smooth muscle around the atria showed more bulk and fluorescence intensity in the AH group compared with the control. The collagen 1 (Col1) plates did not show significant differences except for the abundance of the bright collagen-producing cells seen in the AH group compared with the control. In the merged/DAPI plates (Figs. 6, 7), the collagen-producing cells are seen within parabronchi in the AH group. In addition, some regions of the parenchyma in the AH group exhibit a high DAPI intensity compared to the rest of the tissue. However, at higher magnification, Col1 showed broader distribution in the CT and CH2 groups compared with AH and CH1 groups, and the distribution is less in AH than in CH1. Furthermore, some erythrocytes in AH and CH1 are spherical compared to other groups indicating an increased presence of immature red blood cells in cir culation.

Discussion

This study envisaged that morphological aberrations would occur in the lungs of the Japanese quail exposed to heat stress. Hence, acute and chronic heat exposure was tested using an experimental setup.

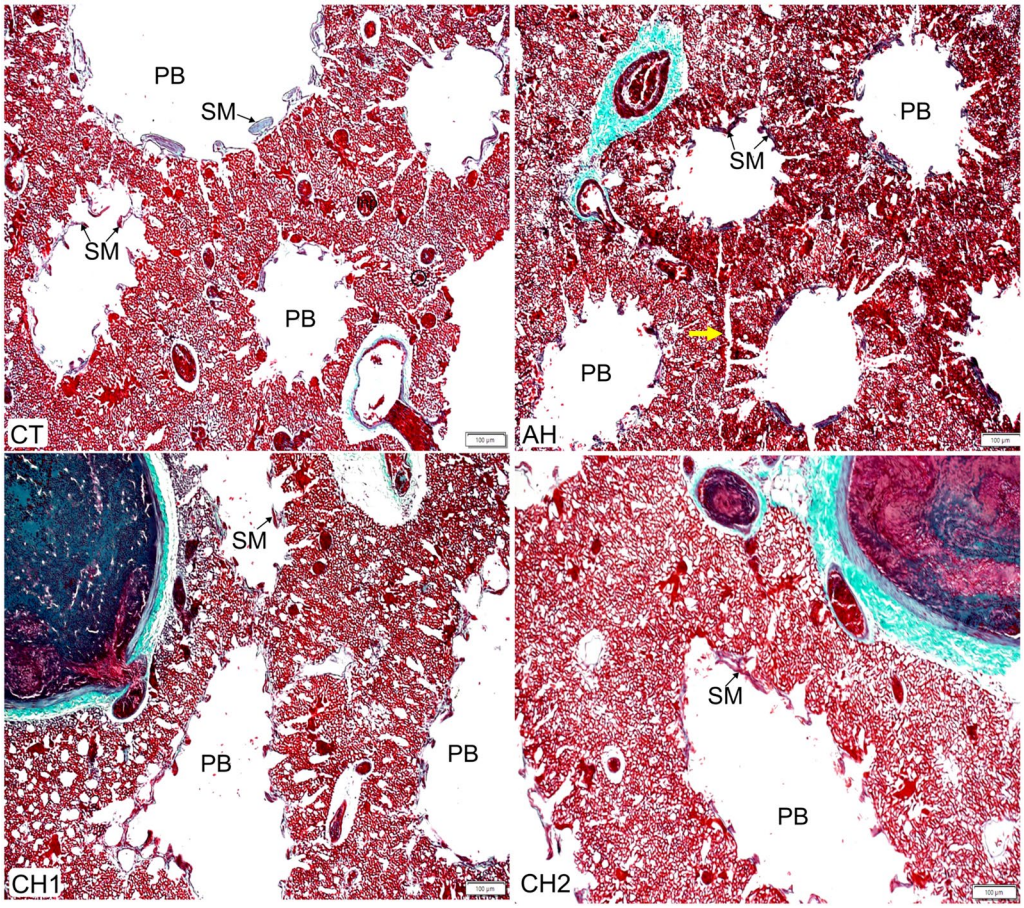

Fig. 3 Representative micrographs of the Gomori's one-step trichrome stained Japanese quail lungs of the control and heat-stressed groups. Yellow arrow indicates area of tissue break in the AH group. PB—the parabronchial lumen; SM—smooth muscle. Scale bar = 100 µm, Mag = 200×

After completing the experiment, no mortality or physical morbidity was recorded in any of the birds. In addition, no significant difference in body weight, cloacal temperature, respiratory rate and the majority of the lung parameters was found in the experimental group compared with the control group. However, the microscopic evaluation of the lung tissue revealed blood congestion, signs of inflammation (increased white cells) and fragility of the tissue in the AH and CH1 groups. However, the CH2 group showed progressive adaptation and recovery.

Significant body weight loss is usually seen with acute heat stress exposure, as reported in the experiment by Del Vesco and Gasparino (2013), in which the Japanese quail were exposed to 34 °C for 24 h. Similarly, in follow-up experiments, significant body weight loss was recorded when the quails were exposed to 38 °C (Del Vesco et al., 2014, 2017). While in chronic heat stress exposure, body weight gain is comparably less than the control group, no weight loss was reported (Mehaisen et al., 2017; Tekce et al., 2020). These results are similar to this study, except that the body weight loss in the AH group—acute heat exposure—was not statistically significant.

The reason for body weight loss in acute heat exposure is the immediate reduction in feed intake by the birds, and there was no time to begin adaptation when the experiment ended, while in chronic heat stress exposure, the duration of the experiment allowed for adaptation and recovery. Hence, this study showed that longer heat exposures had better chances of regaining body weight, depending on the heat intensity. The results from Del Vesco et al. (2017) and this study suggested that the temperature level influences body weight loss once it is above the thermoneutral zone but depends on the duration of exposure and heat intensity. These results also meant that Japanese quails exposed to heat stress are less profitable in business. In addition, outsourcing quails for research could introduce compounding variables if they are exposed to heat stress at the farm.

In addition to reduced feeding, the quail also increases the respiratory rate to enhance heat dissipation through the respiratory surfaces—due to the lack of sweat glands

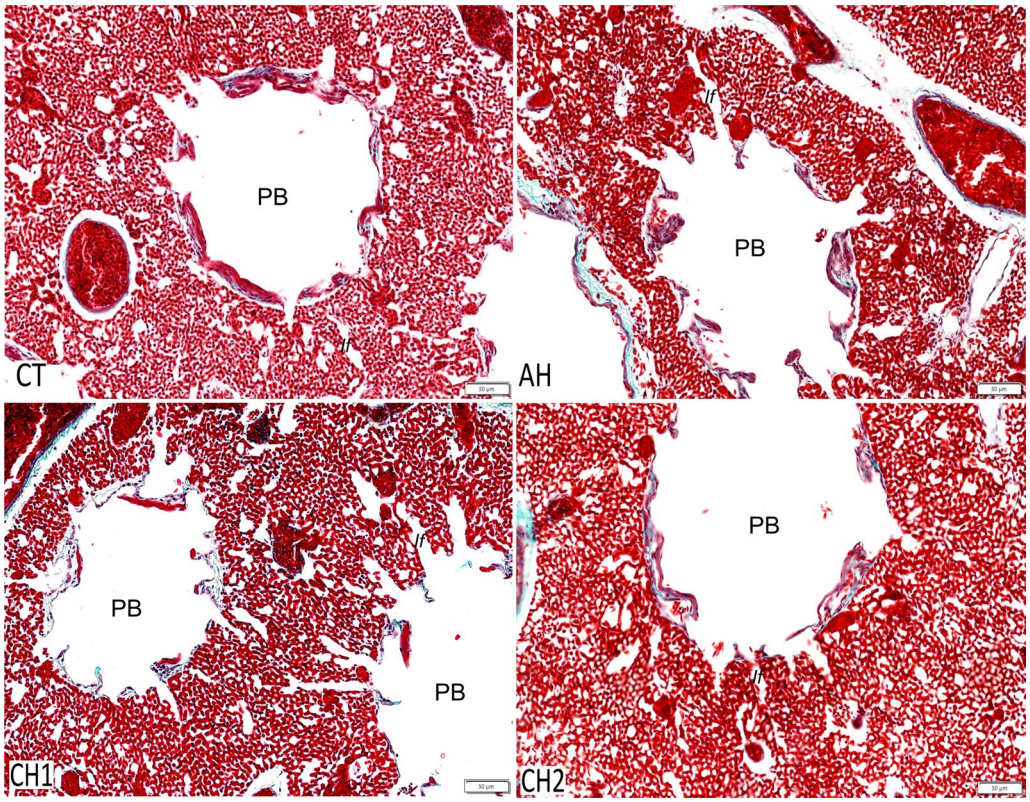

Fig. 4 Representative micrographs of Gomoris' trichrome stained Japanese quail lung of control and heat-stressed groups. Note the blood congestion around the parabronchial lumen (PB) in the AH, CH1 and CH2 groups compared with the control. In addition, the smooth muscles of the interatrial septa show thinning in the CH1 group. If—infundibulae. Scale bar = 50 µm, Mag = 400x

(Marder & Ben-Asher, 1983). Even though there is evidence of cutaneous water evaporation, it is inefficient in Japanese quails compared to birds of similar size (Marder & Ben-Asher, 1983). Therefore, in this study, AH and CH1 groups increased respiratory rates compared with the control, while CH2 had time to adapt and almost revert to regular respiration. Furthermore, the cloacal temperature of the heat-exposed quails was not different in the experimental groups compared with the control group. A slight elevation of the cloacal temperature occurred in the control group. Circadian rhythms' effects on body temperature are the primary suspect for this phenomenon because the infrared bulb turned on and off sporadically based on the drop in chamber temperature. Thus, each time lights are turned on, it affects the rhythm (Woodard & Mather, 1964), and birds become active when they should rest, which raises body temperature (Woodard & Mather, 1964).

Research shows that a 4 °C increase in a bird's body temperature increases the chance of mortality (Saeed et al., 2019). But why would heat stress kill a bird? Some of the reasons in the literature include biochemical derangement (Halici et al., 2012; Sahin et al., 2017), hormonal imbalances (Mehaisen et al., 2019), immune response impairment (Mehaisen et al., 2017) and histological organ damage (Mehaisen et al., 2017). While most of these pathologies were linked to oxidative stress (Akbarian et al., 2016), a direct effect of the hot air could be another reason in the lungs (Marder, 1983). In this study, the lung weight, size and volume reduced in the heat-stressed groups compared with the control. However, while the lung weight was significantly low in the AH group, the lung size and volume were similar to the control.

The fragility noticed in the AH groups' lung tissues could cause decreased organ mass due to loss of structural stability. This is in addition to the increased inflammatory response, which will affect the overall integrity of the lung architecture. The smooth muscles in the parabronchi of heat-exposed quails are more pronounced in the Gomori's trichrome stain and increased in bulk. This is indicating increased activity of myofibrils required to strengthen the overworked smooth muscles; however, the muscles become rigid. The smooth muscles control airflow into the infundibulae and the air capillaries. Hence, increased respiratory rate overwhelms

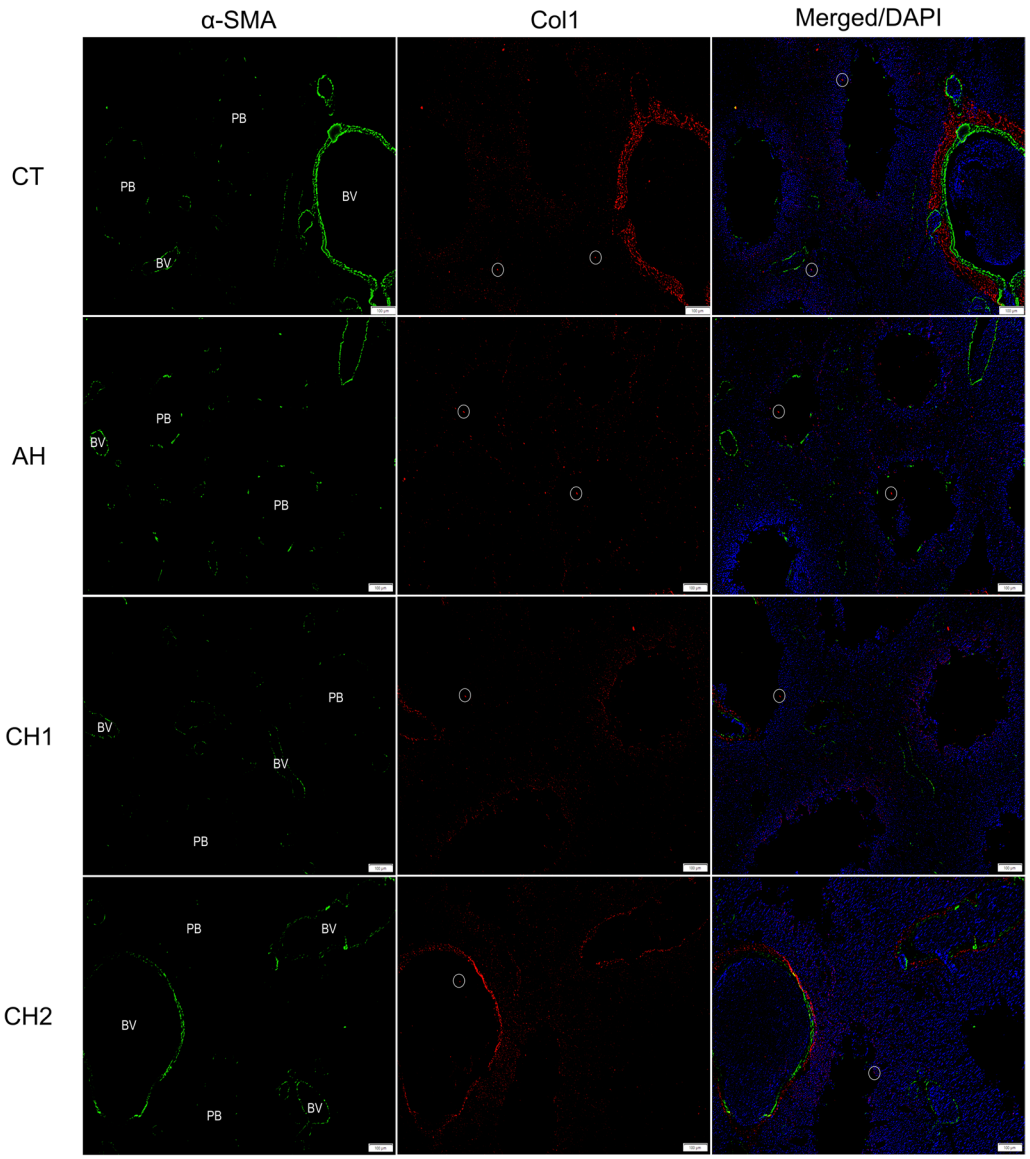

Fig. 5 Representative micrographs of the immunolabelled Japanese quail lungs of the control and heat-stressed groups. Left plates—the anti-α-smooth muscle actin (α-SMA, green) antibody (1:500). Middle plates—the anti-collagen 1 (Col1, red) antibody (1:300). Right plates—the merged micrographs with DAPI (blue) (1:8000). BV—blood vessel; PB—parabronchial lumen; white circles—collagen-producing cells. Scale bar = 100 µm, Mag = 100×

the smooth muscles. The collagen-producing cells in AH and CH1 groups will produce excess collagen fibres in the interatrial septa—an area of high airflow—as part of the inflammatory response (Wynn, 2008). In addition, blood supply increased significantly as an inflammatory response which brought thrombocytes and fibrocytes to the areas of cell damage and injury to initiate repair processes. Furthermore, collagen 1 together with other connective tissue supports the framework of the whole tissue holding the structure together (Wrenn et al., 2018). In this study, we found that heat stress through unknown mechanisms reduces the intensity of collagen 1, thereby weakening the parenchymal integrity.

Therefore, increased collagen deposition targeted the interatrial septa to strengthen it due to increased activity, while the parenchyma is modified to increase gas exchange at the blood-gas barrier. Blood congestion in the lungs of heat-stressed Japanese quail in this study serves the purpose of lung repair and at the same time increased oxygen supply to other body tissues that depend on lung function to avoid anoxia (Rebez et al., 2023). However, the consequence is the rupture of small

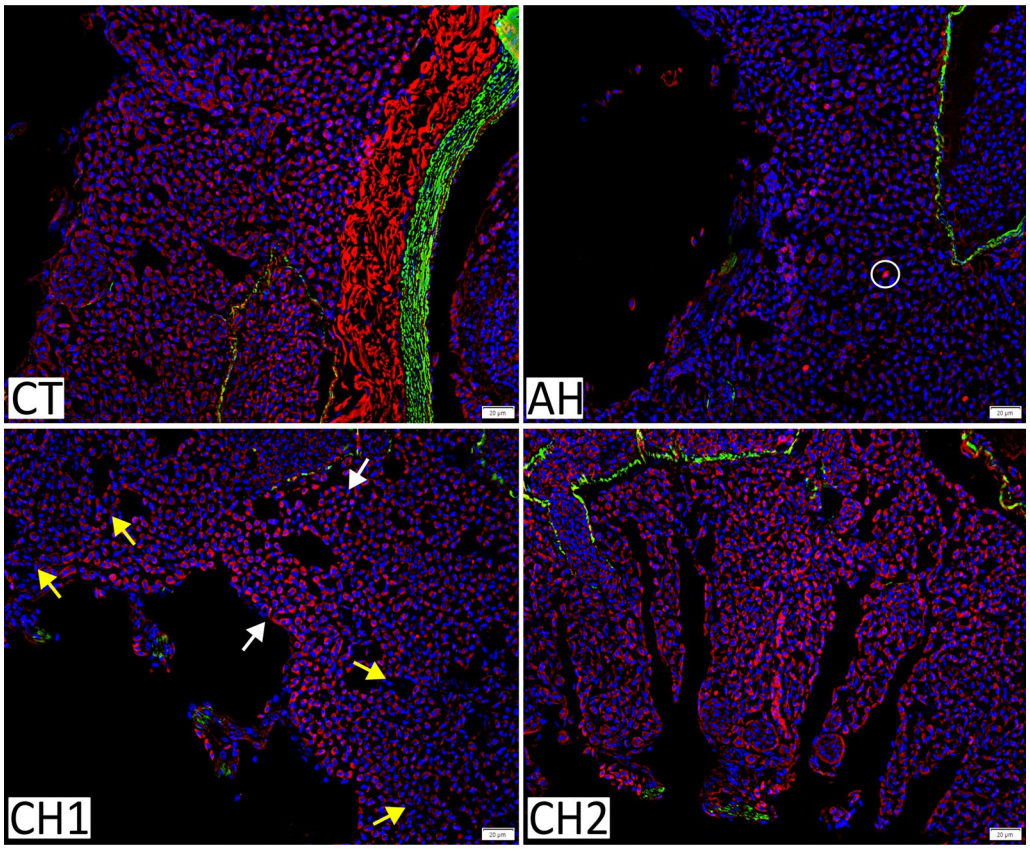

Fig. 6 Representative micrographs of the immunolabelled (merged) Japanese quail lungs of the control and heat-stressed groups. White arrows—regions with high Col1 (red) distribution; Yellow arrows—regions with low Col1 distribution; white circle—collagen-producing cell. In CT and CH2, Col1 is uniformly distributed, while AH and CH1 showed areas of low distribution. Scale bar = 20 µm, Mag = 200x

arteries and arterioles and excessive leakage of blood into the airway.

During normal respiration, a few erythrocytes can leak into the airway, which the surfactants absorb and coagulate (Maina, 2008). However, under heat stress, blood congestion around the parabronchi causes excess leakage as seen in this study and reported in galahs and rock doves exposed to heat stress (Xie et al., 2019). In addition, as shown by Mayer's H&E staining, a large amount of coagulated blood existed in the birds from the AH group. Consequently, immature erythrocytes increase in the heat-stressed groups, indicating the system's response to compensate for the decreased circulatory erythrocytes caused by bleeding and to maintain the increased tissue oxygen demand and support for the repair processes within the lungs.

Heat stress causes a devastating impact on the lungs in the Japanese quail in this study, followed by adaptation, as suggested by the findings in the CH2 group. The morphological changes seen in the AH and CH1 groups must have occurred in the CH2 group, but the CH2 group had a longer duration to adapt and attempt recovery. The long-term implication of heat stress is the energy resources used to adapt, which could be used to grow and reproduce. In this study, the CH2 group had better results than AH and CH1, but still not the same as the control group. We hypothesised that if the stress continues, the quail will continue to adapt till the end of its lifetime (which is short), negatively impacting the quality and quantity of its products.

Another problem is the effect of heat stress on its genes, which could be passed to the next generations and could translate to a phenotype more resilient to heat stress but with a more petite body and weight. High environmental temperature induces body weight reduction over generations, as reported in mountain wagtails (Prokosch et al., 2019). In addition, a model study suggested that global warming will lead to mass avian mortality, especially in smaller birds like the Japanese quail, due to evaporative water loss (McKechnie & Wolf, 2010). Hence, optimal quail production and quality of research specimens require conscious effort to alleviate or remove the effects of heat stress, especially in sub-Saharan rural

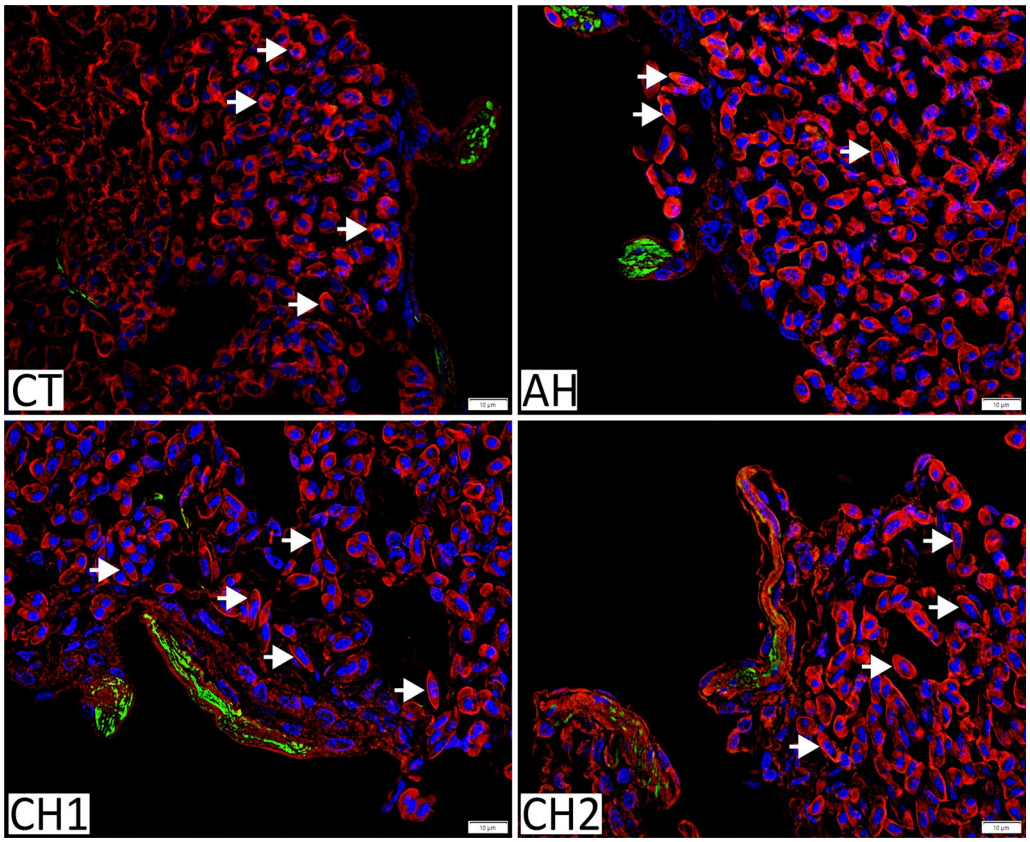

Fig. 7 Representative micrographs of the immunolabelled (merged) Japanese quail lungs of the control and heat-stressed groups. White arrows—erythrocytes. Note the spheric erythrocytes (white arrows) in the AH, CH1 and CH2 compared with ovoid erythrocytes in control (CT). Erythrocytes are seen within the airway in the AH, CH1 and CH2 compared with the control. Scale bar = 10 µm, Mag = 400×

Africa, where it has the potential to provide protein food security (Mnisi et al., 2021).

Conclusions

Heat stress negatively impacted the Japanese quails' lungs through direct effects and oxidative stress. This impact increases the chance of instant mortality or long-term decline in growth and production capacity because other organs depend on the lungs' function to maintain homeostasis. Microclimate to control the environmental temperature and adequate floor space could minimise the effects of heat stress but is expensive. Some cost-effective dietary strategies could alleviate the oxidative stress caused by heat exposure. In addition, the Japanese quail can adapt to heat stress depending on duration and intensity, if it survives the initial impact.

Abbreviations

DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
α-SMA	α Smooth muscle actin
Col1	Collagen 1
DAPI	4′,6-Diamidino-2-phenylindole

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Author contributions

AA and DR contribute equally to conceptualising the idea and conduct of the research, and all authors have read and approved the final manuscript.

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Availability of data and materials

The data are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

This experiment was approved by the Animal Research Ethics Committee of the University of the Witwatersrand, approval number: 2020/10/01/D.

Consent for publication

Not applicable.

Competing interests
The authors declare that they have no competing interests.

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