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Original article

Physiological, haematological and electroencephalographic responses to heat stress in Katjang and Boer goats

Norsam N. Syafiqa [a], Idrus Zulkifli [a,b,*], Abu Bakar Md. Zuki [c], Yoh Meng Goh [a,c], Ubedullah Kaka [d]

[a] Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[b] Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[c] Department of Veterinary Pre-Clinical Science, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[d] Department of Companion Animal Medicine and Surgery, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

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ABSTRACT

The present study aimed to determine the effect of repeated heat stress on serum levels of cortisol (CORT), acute phase proteins (APP) and heat shock protein (HSP) 70, haematological indicators, and electroencephalographic (EEG) response in the native Katjang and exotic Boer goats. Six female Katjang (15.7 kg ± 0.68) and six female Boer (16.8 kg ± 1.16) goats aged 5 to 6 months old were exposed to 38 ± 1℃ for 8 h, and the procedure was repeated at three different weeks (weeks 1, 2 and 3). Measurements of rectal temperatures and EEG activity and collection of blood samples were conducted before heat exposure (0 h), immediately after the heat exposure (8 h), and 8 h after completion of heat exposure (16 h) (recovery period). The current results revealed that the Boer animals had significantly higher rectal temperatures (RT), haemoglobin (Hb) and packed cell volume (PCV) counts than their Kajang counterparts. There were significant breed × stage of heat treatment (SHT) × week of heat treatment (WHT) interactions for neutrophil to lymphocyte ratios (NLR). In general, the Katjang animals had elevated NLR compared to those of Boer. The Boer goats had reduced capacity to express serum HSP70 compared to their Katjang counterparts following the heat challenge at week 3. Boer goats demonstrated higher delta waves than the Katjang group, which suggested the former were more stressed following the heat exposure. Breed had a negligible effect on CORT, APP, WBC counts and backfat thickness. Our findings suggested that the Katjang breed, as measured by RT, HB and PCV count, and EEG activity, could be more tolerant to heat stress than Boer. The Katjang goats showed higher HSP70 expression than their Boer counterparts, suggesting improved thermoregulation in the former.

1. Introduction

The production of goats' milk and meat is a significant component of animal agriculture in many countries and regions. Heat stress problem in goats has recently received more attention because of the expected increases in environmental temperature associated with global warming (Ribeiro et al., 2018). Due to acclimatisation to tropical climates, native goat breeds are typically better thermoregulators in response to higher ambient temperatures than exotic breeds (Silanikove, 2000). Thus, compared to imported breeds, the productivity and well-being of native breeds are less adversely impacted by a hot climate (Sejian et al., 2021).

Goats are more resistant to heat stress than cattle and sheep because of their morphological and physiological characteristics (Sanon et al., 2007). Physical characteristics that favour adaptability include large salivary glands, a larger surface area of absorptive mucosa, and the capacity to expand the foregut with high-fiber diets (Silanikove & Koluman, 2015). Goats' anatomical structure allows for greater body temperature regulation in a hot environment. However, the hot and humid Malaysian weather may induce heat stress in goats. According to Hammadi et al. (2012), the thermoneutral zone of goats lies between 12℃ – 24℃. Malaysia's average maximum and minimum ambient temperatures are 24℃ and 33℃, respectively (Department of Meteorology Malaysia, 2018). Heat stress could be exacerbated by the high relative humidity in the tropics, which impedes evaporative heat loss. Given each breed's unique characteristics, different caprine breeds have different adaptation abilities, with some being more tolerant than others. Breeds that evolved in warm areas regulate their tempera-

* Corresponding author at: Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

E-mail addresses: gs50857@student.upm.edu.my (N.N. Syafiqa), zulidrus@upm.edu.my (I. Zulkifli), zuki@upm.edu.my (Abu Bakar Md. Zuki), dr_ubedkaka@upm.edu.my (U. Kaka).

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ture more successfully than those that evolved in cooler climates when exposed to high ambient temperatures. Due to their inability to consistently adapt to and withstand high ambient temperatures, relative humidity, and solar radiation, high-producing temperate breeds are not always successful when introduced to tropical environments.

In Malaysia, goat breeds are categorised into indigenous, crossbreed, and or imported. Among Malaysia's various domesticated goat breeds, the Katjang goat stands out as the sole indigenous breed (Devendra, 1966). Katjang goats can adapt and adjust to the hot and humid tropical environment. However, the Katjang goats are known for their relatively poor performance compared to other goat breeds (Devendra, 2007). The mature weight is 25 kg for males and 20 kg for females. Boer goats originated from South Africa and are renowned for their superior growth, fertility, and meat quality (Malan, 2000). It is essentially a meat breed but also has good dairy conformation. The mature adult bucks and females weighed 65–90 and 45–65 kg, respectively (Abdul Rashi, 2008). Although the growth performance of local and imported goat breeds in the tropical environment has been well documented, comparative studies on the physiological responses of indigenous and imported goat breeds to heat stress challenges are scarce. It is unclear how these breeds react to heat stress and why breed-level variation exists in their ability to withstand external heat load. Such information is valuable in identifying the most resilient breed that can survive multiple environmental challenges imposed in different production systems. The current study aimed to assess and compare the physiological responses of indigenous (Katjang) and imported (Boer) goat breeds to heat stress. Both breeds significantly differ in body size, shape, and colour. Body size and shape are vital morphological factors impacting farm animals' thermoregulatory systems (Hailey et al., 2019).

Repeated exposure to non-lethal stress may result in adaptation, defined as a progressive reduction in physiological strain in the animal (Taylor 2006). Animal adaptation helps them survive by improving their capacity to withstand stress (Taylor 2006). In response to a thermal challenge, small ruminants adjust to heat-stressed climatic conditions through behavioural, morphological, physiological, and genetic bases (Angilletta 2009). There is, however, no documented work on the ability to adapt to thermal stress in Katjang and Boer goats.

Rectal temperature is commonly used to measure changes in homeostasis imposed by thermal insults in goats (Rashamol et al., 2020). Al-Dawood (2017) indicated that heat stress may evoke changes in haemoglobin (Hb), packed cell volume (PCV), white blood cells (WBCs), neutrophils, and lymphocytes in goats. It is well established that physiological reactions to unpleasant stimuli include activation of the hypothalamic–pituitary–adrenal (HPA) axis (Herman et al., 2016). Blood cortisol levels are the most common method of assessing the HPA function in farm animals. Cellular stress response involving the expression of heat shock proteins (HSP) is also considered a generalised response to noxious stimuli. The induction of HSP in the cells is mainly to sustain many critical physiological functions, such as ensuring stress-denatured proteins' folding, unfolding, and refolding (Archana et al., 2017). In addition, these proteins are activated in cells in response to a range of noxious stimuli. By safeguarding vital biological activities, stresses can be reduced, and survival is improved.

Blood proteins known as acute phase proteins (APPs) change in concentration in response to stress, infections, immunological disorders, and tissue damage (Gabay & Kushner, 1999; Murata et al., 2004). They are primarily produced in the liver by the action of pro-inflammatory cytokines, and their concentration can change depending on whether they are positive or negative APPs. Positive APPs such as haptoglobin and serum amyloid A are potential biomarkers of stress in various species of animals (Cray et al., 2009). Unlike large ruminants (Cray, 2012), published work on APP reaction in goats is limited.

Chang et al. (2005) noted electroencephalographic (EEG) responses to non-painful warm and cold stimuli in human beings. Work in human beings show that administration with exogenous cortisol elicited EEG reaction (Tops et al., 2006; McAllister-Williams et al., 2007). Therefore, non-painful stressful stimuli may increase blood cortisol levels and cause changes in the human EEG. Zulkifli et al. (2019) reported that stress attributed to road transportation elicited EEG reactions in beef cattle. Although differences in thermotolerance between various goat breeds were reported under a hot environment, no study has been conducted to compare physiological responses during experimentally imposed heat stress. Subjecting animals to heat challenges in an indoor environment may eliminate the fluctuating effects of the tropical climate.

The effect of adipose tissue on thermoregulation in the mammalian species has been reported (Nienaber et al., 2004; Gòmez-Prado et al., 2022). An increase in the thickness of the layer of adipose tissue reduces the emission of cutaneous heat into the environment. Therefore, this study compared the response to repeated exposure to acute heat stress between the native Katjang goats and the exotic Boer animals. To achieve this goal, we measured rectal temperature, haematological parameters, HSP 70 and APPs serum levels, EEG activity and backfat thickness.

2. Materials and methodology

2.1. Animal, housing, and husbandry

Six female Katjang (15.7 kg ± 0.68) and six female Boer (16.8 kg ± 1.16) goats were obtained from a local breeder farm in Terengganu. Both breeds of goats were between five to six months old. The local breeder in Terengganu reared the animals in a naturally ventilated, raised, slatted floor house. The goats were transported to the Animal Research Centre, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, by truck. The distance from Terengganu to Universiti Putra Malaysia is 465 km, and the journey took 4 h and 56 min. At Universiti Putra Malaysia, the goats were raised in a mechanically ventilated house with concrete floors that were covered with rice straw as bedding material. The goats were randomly divided into groups of three to four floor pens according to breed when they arrived. The area of each pen was 11.03 m^2. The indoor temperature was 24 ± 1 °C, while the relative humidity ranged from 75 to 80%. According to Lu (1989) and the Temperature and Humidity Index table of South Dakota State University Extension (https://extension.sdstate.edu/heat-stress-small-ruminants), such temperature and relative humidity are within the thermoneutral zone of goats. The thermoneutral environment is vital to obtain baseline values of the various physiological parameters measured. Goats were fed Napier grass and commercial pellets according to 3% (dry matter intake) of their body weights. Drinking water was readily available.

From days 3 to 10 post-arrival, all the goats were transferred to four climatic rooms for six hours daily to allow acclimatisation. It is well documented that exposure to a novel environment may elicit fear and stress reactions in farm animals (Désiré et al., 2002). All three animals from each home pen shared a climatic room [3.57 m (length) × 5.37 m (width) × 2.39 m (height)]. The floor space of each climatic room was 16.60 m^2. The climatic room is mechanically ventilated, and the walls are insulated. The concrete floor is covered with rice straw as bedding material. The temperature in the climatic rooms was set at 24°C, and the relative humidity was 70 – 80%. After 6 h of acclimatisation, the goats were returned to their home pens.

All the goats were transferred to four climatic rooms (3 animals/room) and exposed to 38°C for eight hours on days 11 (WHT1), 18 (WHT2) and 25 (WHT3) post-arrival (Fig. 1). The relative humidity was 53%. The goats' upper critical temperature (UCT) is 25 – 30 °C (Lu, 1989). Bligh and Johnson (1973) indicated that UCT is the ambient temperature above which a physiological heat stress response will be elicited. The maximum temperature in Malaysia can reach as high as 37.3C (Tang, 2019). Thus, 38 °C is a suitable heat challenge temperature to elicit physiological heat stress in goats. Animals were provided drinking water but not fed during the heat treatment. Venipuncture was used to collect blood samples (5 mL) from each goat into tubes with EDTA as the anticoagulant before heat exposure (0 h) (baseline values), immediately after the heat exposure (8 h), and 8 h after completion of heat exposure (16 h) (recovery period). The blood samples at 0 h and 16 h were collected in their respective home pens, while the sampling at the 8 h was conducted in the climatic rooms. An experienced handler restrained the animal individually, and a licensed veterinarian did the blood sampling, according to Mitchell (2023). The restraining and bleeding procedure did not exceed 2 min and should not influence serum cortisol concentrations (Broom and Johnson, 1993).

A thin layer of blood was smeared and stained with methylene blue on a microscope glass slide. The glass slide was then observed and assessed under an electronic microscope. With the help of an automatic haematology analyser (CELL DYN® 3700, Abbott, Lake County, IL, USA), the constituent WBC, PCV, Hb, and neutrophils and lymphocyte counts were ascertained using the Veterinary Package software. All reagents used were from Abbott (Abbott, Lake County, IL, USA. The neutrophil: lymphocyte ratios (NLR) were calculated. Meanwhile, blood samples for hormonal changes were centrifuged at 3000 rpm for 15 min to obtain the plasma and stored at −80°C until assayed. Commercial ELISA kits (Qayee-Bio, Shanghai, China) were used to determine the serum HSP70, haptoglobin (Hp), serum amyloid A (SAA), and cortisol concentrations.

Immediately after the blood sampling at 0 h, 8 h, and 16 h, the rectal temperature of each animal was recorded. The probe was inserted about 3 cm into the rectum for 30 s. Following the body temperature recording, each goat's brain activity was recorded using Power Lab Biopotential Recording Systems (AD Instruments, Bella Vista, NSW, Australia). The targeted area was shaved two days earlier. Two hydrogel conductive adhesive sterile disposal pads (Kendall™, Covidien, Mansfield, MA, USA) were placed on the zygomatic process of the frontal bone and right mastoid process. The inverting (-) and non-inverting (+) electrodes were

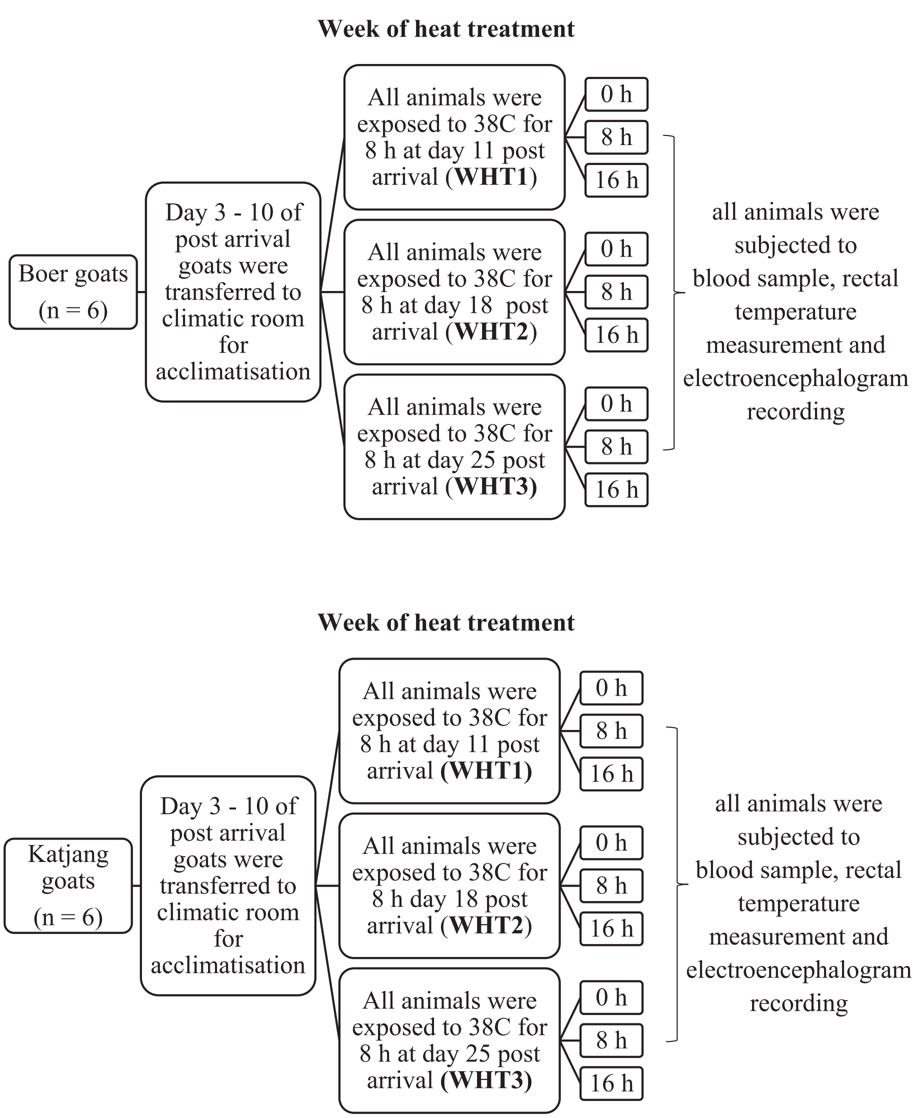

Fig. 1. The experimental design.

attached with a pad to the zygomatic process and mastoid process, respectively. EEG electrode configuration was used on the scalp according to the method described by Gibson et al. (2009). The electroencephalographic signal was recorded at a sampling rate of 1 kHz, and raw EEG was resampled with a low pass filter of 200 Hz into delta frequency (0.1 to 4.0 Hz), theta frequency (4.1 to 8.0 Hz), alpha frequency (8.1 to 12.0 Hz) and beta frequency (12.1 to 20 Hz) as described by Zulkifli et al. (2014). Before EEG analysis, the raw EEG recording was resampled at 1024 Hz, and only frequencies between 0.1 and 30 Hz were obtained to minimise the presence of artefacts. Each animal was subjected to the blood sampling, rectal temperature and EEG recording procedures at three different ages: WHT1, WHT2 and WHT3.

On day 28, following the EEG test at WHT3, the backfat thickness of each animal was measured by ultrasound images using a portable veterinary ultrasound scanner with a 7.5-MHz probe (Explorer V5 Vet Laptop B-Ultrasonic Scanner UMC Technology Development Co., Ltd., China). The measurement was taken between the fourth and fifth lumbar vertebrae. The area was sheared before the measurement was made. Three measurements were recorded, and an average was taken for analysis.

3. Statistical analysis

The Statistical Analysis System (SAS) package Version 9.4 2020 (SAS Institute Inc., Cary, NC, USA) method's PROC UNIVARIATE function was used to determine whether the data were normally distributed. All data except backfat thickness were subjected to analysis of variance for repeated measure analysis using PROC MIXED procedure of Statistical Analysis System (SAS) package Version 9.4 software (SAS Institute Inc., Cary, NC, USA, 2007). The data were analysed with the breed, stage of heat treatment (SHT), and week of heat treatment (WHT) and their interactions were fitted as fixed effects. Means were separated with the Tukey HSD test. One-way ANOVA analysed backfat thickness data with the breed as the only main effect. Values of $p \leq 0.05$ were considered to signify statistically significant differences.

4. Results

4.1. Rectal temperature

There were no significant (p = 0.6906) interactions of main effects for RT (Table 1). The Boer goats had significantly (p = 0.0001) higher RT than their Katjang counterparts. The highest (p < 0.0001) RT measurements were noted at 8 h followed by 0 h and 16 h. The RT at WHT2 and WHT3 of heat treatment, which did not differ, were significantly (p = 0.0706) higher than those at WHT1.

Table 1
Effect of breed, stage of heat treatment, and week of heat treatment on rectal temperature (Mean ± SEM).

Variables	
Item	Rectal temperature (°C)
Breed	
Katjang	38.56 ± 0.11^{b}
Boer	39.19 ± 0.09^{a}
SHT (h)	
0	38.69 ± 0.11^{b}
8	39.59 ± 0.10^{a}
16	38.34 ± 0.10^{c}
WHT	
1	38.63 ± 0.17^{b}
2	39.02 ± 0.12^{a}
3	38.97 ± 0.11^{a}
Source of Variation	
Breed	0.0001
SHT	<0.0001
WHT	0.0706
Breed × SHT	0.7724
Breed × WHT	0.1993
SHT × WHT	0.0881
Breed × SHT × WHT	0.6906

Notes. SHT, stage of heat treatment; h, hour; WHT, week of heat treatment; SEM, standard error of means.
Means within a column subgroup with no common superscripts are different at $p \leq 0.05$.

4.2. Haematological parameters

There were no significant interactions of main effects for Hb (p = 0.9574), PCV (p = 0.7112), and WBC (p = 0.8329) values (Table 2). Katjang goats had significantly lower Hb (p = 0.0053) and PCV (p = 0.0062) counts than the Boer group. Significantly higher Hb (p = 0.0124) and PCV (p = 0.0365) counts were noted in WHT 1 compared to WHT2 and WHT3 of heat treatment. The WBC counts at 8 h were significantly (p = 0.0545) higher than those at 0 h. The WBC counts at 16 h were not significantly (p > 0.05) different from those at 0 h and 8 h. There were significant SHT × WHT (p = 0.0170), and breed × SHT × WHT (p = 0.0161) interactions for NLR.

Except for NLR at 0 h and 8 h during week 2 and week 3, respectively, all the Katjang goats showed similar (p > 0.05) values (Fig. 2). At WHT1, Katjang goats had consistently higher (p < 0.0001) NLR than their Boer counterparts. However, at WHT2, the NLR of Katjang – 0 h goats were similar (p > 0.05) to the Boer group independent of WHT and SHT. Following heat challenge (8 h), a comparison between the two breeds revealed that the NLR of Katjang goats at WHT3 were not significantly (p > 0.05) different from those of Boer – 8 h and Boer – 16 h (week 1), Boer – 0 h and Boer – 16 h (week 2), and Boer – 16 h (week 3).

4.3. Blood cortisol, heat shock protein 70, and acute phase protein concentrations

There were no significant interactions of main effects for CORT (p = 0.8770), SAA (p = 0.2456), and Hp (p = 0.9586) (Table 3). Neither breed (p = 0.0813) nor WHT (p = 0.3257) had a significant impact on CORT. However, the CORT at 8 h were significantly (p = 0.0129) higher than those at 0 h and 16 h. Significant breed × WHT interactions (p = 0.0472) were noted for HSP70. The HSP70 of Boer goats were not significantly (p = 0.3990) affected by WHT (Table 4). However, the Katjang goats had significantly (p = 0.0445) higher HSP70 at week 3 when compared to WHT1. Both breeds showed similar HSP70 at WHT1 (p = 0.3949) and WHT2 (p = 0.9381). On the contrary, the Katjang goats exhibited significantly (p = 0.0331) higher HSP70 than their Boer counterparts at WHT3. Neither breed (SAA, p = 0.4578; Hp, p = 0.6606), WHT (SAA, p = 0.6899; Hp, p = 0.1528), nor SHT (SAA, p = 0.6158; Hp, p = 0.9060), are not significant.

4.4. Electroencephalogram

Delta waves in Boer goats were significantly (p = 0.0177) higher than Katjang (Table 5). Regardless of breed or WHT, the delta waves were significantly (p = 0.0006) affected by SHT. The heat exposure for 8 h increased delta waves compared to recording at 0 h. The delta wave at 16 h was similar (p > 0.05) to those of 0 h and 8 h of heat treatment. All main effects did not significantly influence alpha (breed, p = 0.2099; WHT, p = 0.6848; SHT,

Table 2
Effect of breed, stage of heat treatment and week of heat treatment on haematological parameters (Mean ± SEM).

Variables Item	Hb (g/L)	PCV (L/L)	WBC (x10^9/L)	NLR
Breed				
Katjang	94.23 ± 0.86^b	0.29 ± 0.03^b	14.30 ± 0.36^a	1.81 ± 0.10^a
Boer	99.55 ± 1.65^a	0.31 ± 0.01^a	14.53 ± 0.27^a	0.72 ± 0.05^b
SHT (h)				
0	102.06 ± 1.48^a	0.30 ± 0.01^a	13.74 ± 0.38^b	1.04 ± 0.13^b
8	100.50 ± 1.73^a	0.30 ± 0.01^a	15.11 ± 0.39^a	1.29 ± 0.14^{ab}
16	100.62 ± 1.76^a	0.29 ± 0.01^a	14.40 ± 0.39^{ab}	1.46 ± 0.13^a
WHT				
1	100.70 ± 1.64^a	0.31 ± 0.01^a	14.59 ± 0.27^a	1.43 ± 0.14^a
2	96.03 ± 1.61^{ab}	0.29 ± 0.01^b	14.13 ± 0.37^a	1.21 ± 0.14^a
3	93.94 ± 1.58^b	0.29 ± 0.01^b	14.53 ± 0.49^a	1.15 ± 0.13^a
Source of Variation				
Breed	0.0053	0.0879	0.6239	<0.0001
SHT	0.3191	0.1591	0.0545	0.0045
WHT	0.0124	0.0365	0.6716	0.0680
Breed × SHT	0.5667	0.0763	0.3986	0.3311
Breed × WHT	0.5778	0.9710	0.8237	0.2741
SHT × WHT	0.8333	0.1574	0.9988	0.0170
Breed × SHT × WHT	0.9574	0.7112	0.8329	0.0161

Notes. Hb, haemoglobin; PCV, packed cell volume; WBC, white blood cell; NLR, neutrophil:lymphocyte ratio; SHT, stage of heat treatment; h, hour; WHT, week of heat treatment; SEM, standard error of means.
Means within column subgroup with no common superscripts are different at $p \leq 0.05$.

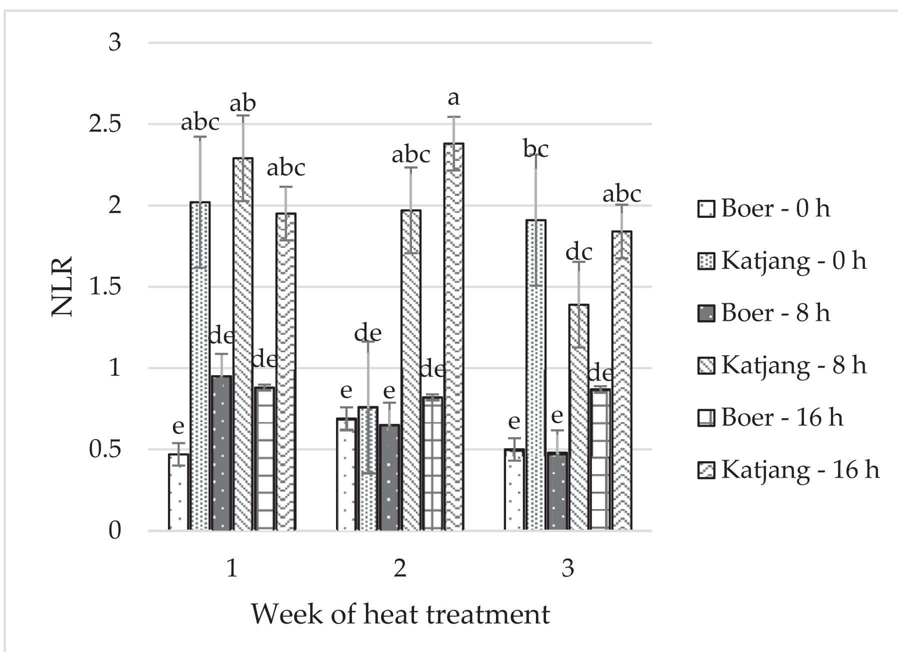

Fig. 2. Significant breed × week of heat treatment × stage of heat treatment interactions for neutrophil:lymphocyte ratio (NLR). Means with no common letters are different at p ≤ 0.05.

p = 0.2492) or beta waves (breed, p = 0.7108; WHT, p = 0.5499; SHT, p = 0.1907).Table 6..

Neither breed (theta, p = 0.0723; total power, p = 0.3169) nor WHT (theta, p = 0.7794; total power, p = 0.7191) had a significant influence on theta waves and the total power of EEG. The theta waves (p = 0.0107) and total power of EEG (p < 0.0001) at 16 h were significantly lower than the reading at 8 h. The MF waves were not significantly (breed, p = 0.6441); WHT, p = 0.5775; SHT, p = 0.1759). affected by any of the main effects. Both Katjang and Boer goats had similar (p = 0.3749) backfat thicknesses (Fig. 3).

5. Discussion

The typical method for determining core body temperature in livestock is rectal temperature. It has been suggested that it is a reliable sign of heat stress because it triggers other heat stress-relieving systems (Gerbremedhin et al., 2008). Change in RT is considered an immediate response to any physiological changes in an animal. In this study, the Boer goats had higher RT than their Katjang counterparts. Katjang is considered an indigenous breed of Malaysia (Devendra, 1987) and can withstand the local climatic

Table 3
Effect of breed, stage of heat treatment and week of heat treatment on serum levels of cortisol, heat shock protein 70, serum amyloid A, and haptoglobin (Mean ± SEM).

Item	Variable CORT (pg/mL)	HSP70 (ng/mL)	SAA (pg/mL)	Hp (ng/L)
Breed				
Katjang	747.33 ± 58.19[a]	334.16 ± 13.98[a]	250.01 ± 10.81[a]	111.86 ± 5.77[a]
Boer	608.77 ± 43.76[a]	278.05 ± 7.94[b]	221.01 ± 18.56[a]	107.67 ± 7.02[a]
SHT (h)				
0	589.08 ± 59.07[b]	262.88 ± 13.32[b]	232.42 ± 18.49[a]	107.07 ± 7.84[a]
8	842.50 ± 62.18[a]	377.16 ± 27.88[a]	238.98 ± 19.60[a]	111.23 ± 8.06[a]
16	611.71 ± 64.67[b]	279.05 ± 22.71[b]	235.13 ± 18.41[a]	110.00 ± 7.81[a]
WHT				
1	603.45 ± 51.15[a]	271.51 ± 12.78[b]	214.83 ± 15.12[a]	96.60 ± 6.88[a]
2	705.69 ± 57.31[a]	306.94 ± 15.93[ab]	235.95 ± 13.43[a]	116.61 ± 8.16[a]
3	731.62 ± 80.29[a]	341.85 ± 9.97[a]	255.75 ± 25.16[a]	116.10 ± 8.14[a]
Source of variation				
Breed	0.0813	0.0197	0.4578	0.6606
SHT	0.0129	0.0003	0.6158	0.9060
WHT	0.3257	0.0588	0.6899	0.1528
Breed × SHT	0.9157	0.5600	0.5432	0.9341
Breed × WHT	0.7110	0.0472	0.7761	0.8940
SHT × WHT	0.8548	0.1768	0.1000	0.8051
Breed × SHT × WHT	0.8770	0.1300	0.2456	0.9586

Notes. CORT, serum levels of corticosterone; HSP70, serum levels of heat shock protein 70; SAA, serum levels of serum amyloid A SHT; Hp, serum levels of haptoglobin; SHT, stage of heat treatment; h, hour; WHT, week of heat treatment; SEM, standard error of means.
Means within a column subgroup with no common superscripts are different at $p \leq 0.05$.

Table 4
Mean (±SEM) serum levels of heat shock protein 70 where week of heat treatment × breed interactions were significant.

Breed	WHT 1	2	3
Katjang	286.19 ± 18.52[bx]	308.25 ± 21.65[abx]	412.37 ± 58.79[ax]
Boer	256.84 ± 28.59[ax]	305.62 ± 25.68[ax]	271.32 ± 23.52[ay]

Notes. WHT, week of heat treatment; SEM, standard error of means.
[a,b]Means within a row subgroup with no common superscripts are different at $p \leq 0.05$.
[x,y]Means within a column subgroup with no common superscripts are different at $p < 0.05$.

conditions (Ernie Muneerah et al., 2021). Indigenous breeds thrived well in poor forage and stressful situations compared to imported and crossbred animals (Akinyi, 2008). The smaller body size of the Katjang goats compared to their Boer counterparts is an advantage for tolerating hot climates because of the animals' reduced feed and water needs. Physical changes are a morphological adaptation that occurs over many generations of animals to fit each environmental situation (Sejian et al., 2018). According to Chedid et al. (2014), fat storage is a central morphological adaptation in small ruminants other than body size and shape, coat and skin colour, and hair type. In the present study, both Katjang and Boer had similar backfat thickness. Nienaber et al. (2004) indicated that heat stress tended to reduce backfat thickness in pigs which is beneficial for lower maintenance requirements.

As expected, the 8 h of heat challenge elevated the RT of animals. Interestingly, the RT following recovery from heat challenge (16 h) was lower than at 0 h. Feed withdrawal and increased water intake during the heat challenge period may have lowered animals' body temperature during the recovery period (Sejian et al., 2018). The observed lower RT of goats at week 1 than those at week 2 and 3 is unexpected. It was hypothesised that a certain magnitude of adaptation to the heat challenge could be achieved at week 2 or week 3. However, the converse was noted. There is no clear explanation for the findings. Although the animals gained weight from

Table 5
Effect of breed, stage of heat treatment, and week of heat treatment on alpha, beta, and delta waves (Mean ± SEM).

Item	Alpha (μV)	Beta (μV)	Delta (μV)
Breed			
Katjang	0.93 ± 0.04[a]	1.64 ± 0.08[a]	5.28 ± 0.18[b]
Boer	1.01 ± 0.02[a]	1.56 ± 0.04[a]	6.01 ± 0.17[a]
SHT (h)			
0	0.99 ± 0.04[a]	1.65 ± 0.07[a]	5.15 ± 0.16[b]
8	0.98 ± 0.04[a]	1.59 ± 0.09[a]	6.17 ± 0.24[a]
16	0.93 ± 0.04[a]	1.55 ± 0.08[a]	5.65 ± 0.24[ab]
WHT			
1	0.99 ± 0.04[a]	1.70 ± 0.09[a]	5.92 ± 0.26[a]
2	0.94 ± 0.04[a]	1.58 ± 0.07[a]	5.51 ± 0.22[a]
3	0.97 ± 0.04[a]	1.50 ± 0.06[a]	5.51 ± 0.18[a]
Source of variation			
Breed	0.2099	0.7108	0.0177
WHT	0.6848	0.5499	0.4007
SHT	0.2492	0.1907	0.0006
Breed × SHT	0.4152	0.8596	0.6514
Breed × WHT	0.2869	0.6860	0.3940
SHT × WHT	0.5151	0.0620	0.6895
Breed × SHT × WHT	0.5903	0.4412	0.1669

Notes. SHT, stage of heat treatment; h, hour; WHT, week of heat treatment; SEM, standard error of means.
Means within a column subgroup with no common superscripts are different at $p \leq 0.05$.

weeks 1 to 3, it has been shown that heavier animals are less tolerant to heat stress (Renaudeau et al., 2012).

Blood profiling is an essential indicator of physiological changes when animals are subjected to any stressful event. Resisting against and coping with environmental stimuli is the body's primary defence mechanism (Das et al., 2016). The leading indicators of immunity included Hb, PCV, WBC, and NLR. The PCV and Hb values at 0 h, 8 h, and 16 h were similar in the present study. Thus,

Table 6
Effect of breed, stage of heat treatment and week of heat treatment on theta, total power of EEG, and median frequency waves (Mean ± SEM).

Item	Theta (μV)	Total power of EEG (μV)	MF (μV)
Breed			
Katjang	1.47 ± 0.06[a]	9.24 ± 0.27[a]	19.97 ± 1.35[a]
Boer	1.64 ± 0.04[a]	9.67 ± 0.26[a]	21.44 ± 1.68[a]
SHT (h)			
0	1.59 ± 0.06[ab]	8.60 ± 0.22[b]	22.52 ± 2.05[a]
8	1.74 ± 0.07[a]	11.16 ± 0.38[a]	19.05 ± 1.82[a]
16	1.51 ± 0.06[b]	8.72 ± 0.23[b]	20.61 ± 1.72[a]
WHT			
1	1.59 ± 0.06[a]	9.23 ± 0.29[a]	18.68 ± 1.57[a]
2	1.53 ± 0.07[a]	9.68 ± 0.45[a]	20.49 ± 2.19[a]
3	1.55 ± 0.06[a]	9.52 ± 0.25[a]	23.04 ± 1.89[a]
Source of variation			
Breed	0.0723	0.3169	0.6441
WHT	0.7794	0.7191	0.5775
SHT	0.5107	<0.0001	0.1759
Breed × SHT	0.6953	0.5636	0.1987
Breed × WHT	0.1216	0.3061	0.7000
SHT × WHT	0.2015	0.6684	0.9606
Breed × SHT × WHT	0.2963	0.9974	0.5998

Notes. SHT, stage of heat treatment; h, hour; WHT, week of heat treatment; SEM, standard error of means.
Means within a column subgroup with no common superscripts are different at $p \leq 0.05$.

heat challenge appears to have a negligible impact on both haematological parameters. In their review of the literature, Gupta and Mondal (2021) indicated that heat stress had an inconsistent effect on PCV and Hb in small ruminants. Singh et al. (2016) reported that values of Hb and PCV in sheep were reduced during the hot summer due to erythropoiesis. On the contrary, Sejian et al. (2013) noted elevations in Hb and PCV values in sheep exposed to short-term heat stress. The negligible influence of a hot environment on PCV (Yousif, 2019) and Hb (Al-Haidary, 2004; Srikandakumar et al., 2003) values in small ruminants have also been reported. Both PCV and Hb values are closely associated with hydration status (Boyd, 1981). Because drinking water is readily available during the heat challenge period, the animals may not be dehydrated in the present study. Unlike the current work, previous studies (Al-Haidary, 2004; Alam et al., 2011) suggested elevated PCV and Hb in chronically heat-stressed-small ruminants. Higher PCV and Hb values in Boer goats compared to their Katjang counterparts could also be associated with breed differences. Significant variations in haematological variables between goat breeds have been documented (Gupta & Mondal, 2021).

In the present study, the WBC counts were significantly higher following 8 h of heat treatment than baseline (0 h) and during the recovery period (16 h). The present results concur with Ellamie et al. (2020) that heat stress may result in leucocytosis in small ruminants. During stress, elicitation of the hypothalamic–pituitary–adrenal axis may result in leukocytosis (Pathipati et al., 2020). Stress may modify neutrophil and lymphocyte counts and it can be directly associated with stress hormones (Davis et al., 2008). Studies in ruminants (O'Loughlin, 2011), pigs (Sutherland et al. 2006), and poultry (Scanes, 2016) show increases in the numbers of neutrophil or heterophil and decreases in lymphocyte numbers in response to stress and glucocorticoid treatment. The relative proportion of neutrophils to lymphocytes is frequently considered a good physiological indicator of stress in mammalian and avian species since the number of neutrophils and lymphocytes is influenced by stress in different directions (Johnstone et al. 2012; Scanes, 2016) species. NLR is regarded as a better measure of stress than N or L counts because the ratio is less variable (Davis et al., 2008). Significant breed × SHT × WHT interactions were noted for NLR. In general, the Katjang goats had higher NLR compared to their Boer counterparts. Thus, it appears that the Katjang goats were more distressed than the Boer animals. At weeks 1 and 3, the Katjang goats had higher NLR than the Boer group at 0 h. There is no clear explanation for the elevated NLR in the Katjang goats at 0 h. Both Katjang and Boer animals were exposed to a thermoneutral environment in a mechanically ventilated house and appeared healthy before the heat challenge. The blood sampling for haematological parameters was conducted 11 days post-arrival, and thus both breeds of goats should have recovered from the stress attributed to road transportation. Minka and Ayo (2013) transported goats for 12 h and noted that the animals' metabolism, behaviour, and body weight recovered after 7 – 11 days. In the present study, the animals were exposed to a novel environment and social mixing, which can be stressful (Miranda-de la Lama et al., 2012). The authors indicated that while NLR was unaffected by the novel environment and social mixing, circulating cortisol was elevated in lambs sampled on days 1 and 7, compared with days

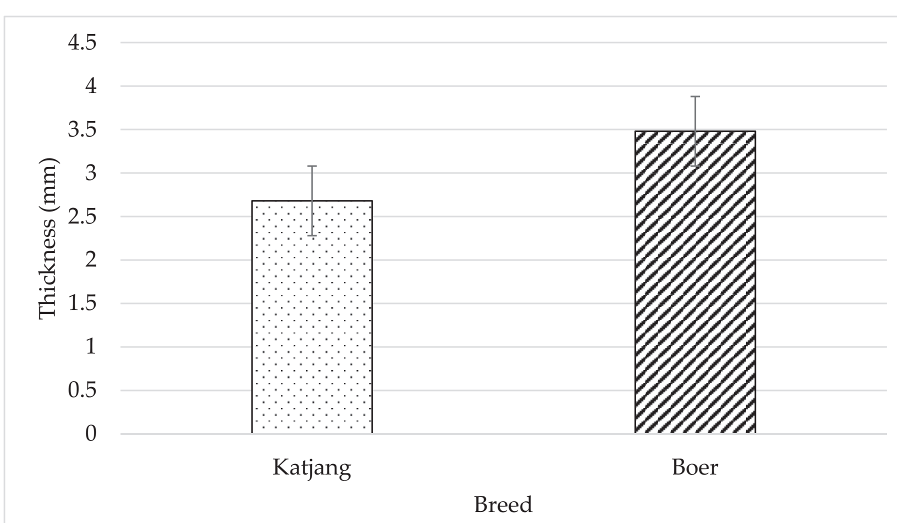

Fig. 3. Effect of breed on backfat thickness. Means are not significantly different (p < 0.05). Six does per breed were sampled.

14 and 28. There is a possibility that the Boer goats could better adapt to the novel housing and social mixing than their Katjang counterparts. At WHT 1 and 2, as measured by NLR at 8 h, the Katjang were more susceptible to heat stress than the Boer group. However, on a cautionary note, the Katjang goats had higher NLR than their Boer animals before the heat challenge (0 h) at WHT1. The elevated NLR exhibited by the Katjang goats at 8 h compared to their Boer counterparts could be a carry-over effect from 0 h.

Exogenous treatment with stress hormones elicited neutrophilia and lymphocytopenia in both mammalian and avian species (Davis et al., 2018). However, in the present study, the CORT data did not concur with those of NLR. Irrespective of SHT and WHT, although the Katjang goats showed higher CORT than Boer, this difference did not quite achieve statistical significance ($P < 0.0813$). In their review, Davis and Maney (2018) concluded that although acute heat stress altered both circulating levels of corticosteroids and NL, they were not consistently correlated at baseline and hence were not interchangeable. The authors suggested that these discrepancies are caused by the nature of the stress reaction and the function of cortisol in contrast to leucocytes during the stress response.

As expected, the heat challenge for 8 h elevated CORT. These findings confirmed earlier work that heat stress elicited CORT response in goats (Sejian & Srivastava, 2010). Sharma et al. (2013) showed an increased plasma level of cortisol in Barbari goats subjected to 35 and 40 °C for 6 h daily for 6 days. However, Kruger et al. (2016) exposed South African indigenous goats to direct sunlight for three hours with an ambient temperature exceeding 30 °C and noted no changes in blood cortisol levels. These discrepancies suggest that variations in temperature, duration of exposure, and breed may influence circulating cortisol response to heat stress.

The current findings show that the heat challenge had a negligible effect on SAA and Hp. Al-Dawood, (2017b) exposed Baladi (an indigenous breed of Jordan) goats to direct sunlight for 14 h per day for 7, 14, 21, and 28 days and noted significant increases in SAA and Hp serum levels of serum amyloid A and haptoglobin. Working with dairy goats, Hamzaoui et al. (2013) showed that exposure to 37 °C for 12 h and 30.5 °C for 12 h elevated blood haptoglobin levels. Hence, the discrepancies in the present findings and those of Al-Dawood, (2017a) and Hamzaoui et al. (2013) could be attributed to variations in the heat exposure duration. Najafi et al. (2016) showed that serum levels of APPs (alpha-1 acid glycoprotein, ceruloplasmin, and ovotransferrin) were elevated after 30 h of feed deprivation in broiler chickens. The maximum blood APPs are commonly attained within 24–48 h following exposure (Jain et al., 2011).

Heat shock protein 70 is considered the most sensitive HSP to heat and plays a significant role in thermoregulation by folding proteins and refolding misfolding proteins to prevent protein aggregation (Kregal, 2002). Our findings and those of Aleena et al. (2018) showed that heat stress elicited HSP70 expression in small ruminants. HSP70 responses are considered a cellular thermometer because there is clear evidence that the synthesis of HSP70 is temperature-dependent (Zulkifli et al., 2003). Following heat exposure, there is an increase in HSP70 expression related to the stimulation and rapid induction of HSP70 mRNA transcription and HSP70 protein translation to protect cells from heat stress. The current work shows that the level of HSP70 returned to baseline value after eight hours of recovery from the heat challenge period. (Collier et al., 2006). Working with heat-stressed goats, Rout et al. (2016) compared HSP70 expression in various tissues and concluded that the highest concentrations were found in the liver, kidney, and heart, followed by the brain, spleen, lungs, and testis. This study is the first to show the presence of HSP in stressed goats' blood circulation. Heat-evoked HSP70 response in various sheep breeds has been shown (Akinyemi et al., 2019).

Although HSP70 has been linked with higher temperature stress in small ruminants (Hyder et al., 2017), it is unknown whether HSP expression is differential between native and imported breeds under heat stress. Significant breed × WHT interactions were noted in the current study for HSP70 expression. It appears that the ability of Katjang goats to induce HSP70 response to heat exposure was enhanced at week 3 (the third episode of the heat challenge). The HSPs mRNA transcription may have been increased by earlier heat challenge episodes, but the RNA has been "sequestered" and not translated until exposure to a later heat challenge at week 3 (Kregel, 2002; Zulkifli et al., 2002). However, the HSP70 of Boer goats was not affected by WHT. It was also noted that the Katjang goats had higher HSP70 than their Boer counterparts at week 3. Breed differences in HSP response at protein (Akinyemi et al., 2019; Aleena et al., 2018) and mRNA levels (Rout et al., 2016) in heat-stressed small ruminants have been demonstrated. The native Katjang goats can better express HSP70 following heat challenges than their exotic Boer counterparts. The high expression of HSP70 in the Katjang animals compared with the Boer group is crucial for resistance to stress and adaptation, which affects the ability to cope with heat stress. On a cautionary note, however, overexpression of HSP may be biologically costly as it could be detrimental to growth and fertility (Sørensen et al., 2003).

Zulkifli et al. (2019) reported that non-painful stressful stimuli elicited blood cortisol and EEG reactions in road-transported Brahman cattle. We report for the first time EEG response to non-pain stimulus in goats. There were four band wave recorded during the EEG recording, which is the low-frequency delta and theta, and high-frequency alpha and beta. Delta and theta oscillations suggest sleepiness, alpha oscillations indicate relaxation, and beta oscillations indicate greater brain activity. According to Schomer and Da Silva (2012), in animals, beta waves are associated with the state of stress and fear, delta waves represent sleep and unconsciousness. In contrast, alpha waves are associated with a non-stressed and relaxed state. In the present study, irrespective of breed, theta and total EEG power at 8 h were significantly higher than those at 0 h. Hence, it appears that the stress associated with the heat challenge has elicited EEG responses in goats. Theta wave frequency has been closely related to emotional excitement in rabbits (Yamamoto, 1998). Work in pigs (Rault et al., 2019) suggested that stress due to isolation and human presence increased the total power of EEG while pleasant human contact reduced the wave. The findings showed that Boer goats demonstrated higher delta waves than the Katjang group, suggesting the former were more stressed after the heat exposure.

The objective of the current study was to compare the responses of the native breed Katjang with the imported breed Boer to repeated heat treatment. The Katjang goats, as measured by RT, PCV and Hb values, and EEG response, were more heat-tolerant than their Boer counterparts. The higher resistance to heat stress of Katjang goats could be attributed to the enhanced expression of HSP70 compared to the Boer animals. The noted augmented HSP70 expression and higher NLR in Katjang goats under heat stress were paradoxical. However, in their review, Davis et al. (2008) questioned whether heterophilia indicates stress and illness (and therefore low fitness) or whether it indicates a superior ability to respond to infection (high fitness). Similarly, the authors indicated that lymphopenia may suggest an active stress response, lack of parasites, or immunosuppression. Thus, the interpretation of NLR in animals remains contentious, and more significant effort should be made further to elucidate the underlying reasons for leucocyte changes in ruminants. There is little evidence, as measured by data at WHT1, WHT2 and WHT3, to suggest that repeated exposure to heat challenge elicits adaptation in Katjang and Boer goats.

6. Conclusion

The current study shows significant differences in the physiological and EEG responses between the two breeds of goats under heat-stress conditions. Most parameters suggest that the Katjang breed has a more robust tolerance to heat stress than their Boer counterparts. Besides the morphological and body size factors, the better able to express HSP70 during heat challenges may have accounted for the superior heat tolerance in the Katjang breed. However, further experiments are required to determine the exact role of HSP70 in conferring heat tolerance in the Katjang goats.

7. Animal welfare statement

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia: UPM/IACUC/AUP-R004/2018.

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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