ORIGINAL PAPER

CrossMark

Acute heat stress induces changes in physiological and cellular responses in Saanen goats

Henrique Barbosa Hooper[1] · Priscila dos Santos Silva[1] · Sandra Aparecida de Oliveira[1] ·
Giovana Krempel Fonseca Merighe[1] · João Alberto Negrão[1]

Abstract

The relationships between rectal temperatures and physiological and cellular responses to heat stress can improve the productivity of Saanen goats in tropical environments. In this context, this study evaluated the physiological responses and gene expression of heat shock proteins (HSP60, 70, and 90) and genes related to apoptosis (Bax, Bcl-2, and p53) of Saanen goats subjected to acute heat stress. Ten health Saanen goats were exposed to solar radiation during 3 consecutive days. The expression of HSP60, HSP70, HSP90, Bax, Bcl-2, and p53 genes in blood leukocytes, rectal and superficial temperatures, respiratory frequency, cortisol, triiodothyronine, and thyroxine was measured at 06:00, 13:00, and 18:00 h. In vitro, blood leukocytes were subjected to 38 °C and 40 °C for 3 h to measure the expression of the same target genes. The temperature humidity index, measured from 12:00 to 15:00, was greater than 80 and black globe temperatures were greater at 40 °C, indicating the intensity of the solar radiation. Although the solar radiation caused acute heat stress, increased cortisol release, and the expression of HSP60 and 70 in dry Saanen goats, the increased respiratory frequency and decreased T4 and T3 restored the homeothermy of the experimental goats. In vitro, the 40 °C increased the expression of p53 (pro-apoptotic protein), Bcl-2 (anti-apoptotic protein), HSP60, HSP70, and HSP90, suggesting that these genes have protective functions. However, further studies are necessary to understand the physiological and cellular responses to heat stress.

Keywords Cortisol · Bax · Bcl-2 · p53 · HSP60 · HSP70

Abbreviations

°C	Degrees Celsius
bpm	Breath movements per minute
Bax	Bcl-2-associated X protein
Bcl-2	B cell lymphoma 2
Cort	Serum cortisol concentration
FZEA	Faculty of Animal Science and Food Engineering
h	Hours
HSPs	Heat shock proteins
min	Minutes
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
p53	Protein 53
RF	Respiratory frequency
RH	Relative humidity
T3	Triiodothyronine
T4	Thyroxine
AT	Air temperature
BGT	Black globe temperature
DT	Dorsal temperature
DPT	Dew point temperature
THI	Temperature and humidity index
MT	Mammary gland temperature
OT	Ocular temperature
RT	Rectal temperature
TT	Tail temperature
USP	University of São Paulo
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
mK	Millikelvin

✉ João Alberto Negrão
jnegrao@usp.br

1 Laboratory of Animal Physiology, Department of Basic Science, Faculty of Animal Science and Food Engineering, University of São Paulo, Duque de Caxias Norte 225, Pirassununga, São Paulo 13635-900, Brazil

Introduction

Farm ruminants are constantly exposed to environmental factors and climate challenges that could cause stress and negatively

Springer

impact their production and reproduction (Minton 1994; McMichael et al. 2007; Narayan and Parisella 2017; Narayan et al. 2018). In tropical latitudes, ruminants are maintained on grassland pasture and are exposed, most of the time, to intense solar radiation. In general, goats from hot climates are less susceptible to solar radiation, water deprivation, lower forage availability, and heat stress (Brasil et al. 2000; de Silva et al. 2006). Goats are a good alternative to conventional animal production in hot climatic condition due to their ability to adapt to different environments (Silanikove 2000; de Silva et al. 2006). During heat stress, however, goats increase their rectal temperature, respiratory frequency, and sweating to change their body heat and show high cortisol (Cort) and low tyrosine (T4) and triiodothyronine (T3) concentrations to maintain their homeothermy (Maia et al. 2015; Ribeiro et al. 2016).

In the same way, heat stress also triggers cellular responses and induces transcription and translation of several genes and changing protein synthesis (Ohtsubo et al. 2001; Johnson et al. 2006; Bernabucci et al. 2010). The overexpression of heat shock proteins (HSPs) in the blood leukocytes of Barbari goats in hotter seasons suggests a protective role of HSPs for maintaining cell integrity (Dangi et al. 2012). Additionally, other authors have suggested that HSP60, HSP70, and HSP90 have essential roles in cell survival by preventing apoptosis during different stress events (Lanneau et al. 2007; Hartl et al. 2011; Hu et al. 2016). In contrast, p53 is a key regulator of apoptosis and both Bcl-2 (anti-apoptotic) and Bax (pro-apoptotic) proteins also have important roles in the regulation of cell survival (Chen and Chuang 1999; Du et al. 2008; Gu et al. 2014). In fact, cell survival depends of the synthesis and accumulation of protective (HSPs, Bcl-2) or apoptotic proteins (p53 and Bax).

In this context, the relationships between physiological and cellular responses to heat stress can improve the effectiveness of genetic selection of Saanen goats for tropical climates (Paula-Lopes et al. 2003; McManus et al. 2009; Pereira et al. 2014) because goats mobilize thermoregulatory responses to maintain their homeothermy during heat stress. Thus, the information on Saanen goats that originated from temperate latitudes and bred for several generations in tropical latitudes is scarce. The objective of this study was to evaluate the relationships between body temperatures, physiological responses, and cellular responses in non-lactating Saanen goats exposed to acute heat stress caused by solar radiation exposure on thermoregulatory responses, Cort, T4 and T3 concentrations, and the expression of HSP60, HSP70, HSP90, p53, Bcl-2, and Bax in vivo and in vitro.

Materials and methods

Experimental location

The present study was carried out at the Animal Physiology laboratory, Animal Science and Food Engineering Faculty (FZEA), University of São Paulo (USP), Pirassununga (21° 58′ S and 47° 26″ W), Brazil. The climate is classified, according to the Köeppen-Geiger classification, as subtropical humid. All animal procedures were in compliance with established ethical procedures and were approved by the ethic committee of the FZEA/USP.

Weather data

Air temperature (AT), black globe temperature (BGT), and relative humidity (RH) were recorded by a data logger (Onset HOBO®, Cape Cod, MA, USA) during the experimental period. The influence exerted by the environment on the animals was described using the temperature and humidity index (THI) and calculated as described by Thom (1959): $THI = AT + 0.36 \times DPT + 41.5$, where AT is the air temperature and DPT is the dew point temperature, both in degrees Celsius. As previously reported (Silanikove and Koluman 2015), the THI was classified as normal when lower than 80, alert when ≤ 80 and < 85, danger when ≤ 85 and < 90, and extreme when ≥ 90.

The details concerning AT, BGT, RH, and THI from 06:00 am to 18:00 pm are shown in Table 1. During the in vivo study, the maximum AT (33.20 °C) and BGT (43.88 °C) were recorded at 14:00 and 13:00 h respectively, indicating the intensity of solar radiation imposed on the goats. The discomfort or stress of the experimental goats was measured by the THI and the THI measured from 12:00 to 15:00 was classified as the alert condition because the THI was above 80 (Table 1).

Animals, pens, and diets

Ten healthy, female dry 5-year-old Saanen goats with an average body weight of 46.2 ± 5.6 kg were used in the present study. Experimental goats were maintained in collective covered pens with free access to shade, feed, water, and mineral salt in collective troughs. Experimental goats received a total diet (corn silage and mixed grains at 60:40) twice a day (at 08:00 am and 16:00 pm). Taking into account the age, category, body weight, and body condition score, the diet was adjusted to provide 100% of the animal's dietary needs (NRC 2007) and feed intake was adjusted daily with 10% of leftovers.

Experimental procedures

In the present study, two experiments were performed, one in vivo and another in vitro. In the in vivo experiment, the Saanen goats were exposed to solar radiation during 3 typical days of spring in an area without any type of shade ($420 \, m^2$ of total area, fenced, smooth concrete floor with feeder, and water troughs), where they remained from 07:00 am to 17:00 pm (experimental challenge) but with access to feed and water.

Table 1 Mean ± standard error of mean of air temperature (AT), black globe temperature (BGT), relative humidity (RH), and temperature humidity index (THI) by hour measured during the in vivo experiment

Item	Hours													P value
	06:00*	07:00	08:00	09:00	10:00	11:00	12:00	13:00*	14:00	15:00	16:00	17:00	18:00*	
AT (°C)	19.59[c]	21.31	23.66	25.33	27.49	28.99	31.00	32.05[a]	33.20	31.14	28.82	28.02	26.63[b]	< 0.001
BGT (°C)	19.25[c]	21.85	24.67	26.37	29.53	32.07	34.04	40.93[a]	43.88	35.23	30.75	29.22	25.51[b]	< 0.001
RU (%)	87.53[a]	84.13	76.12	70.13	64.31	61.22	60.20	51.36[c]	47.47	49.47	60.87	63.23	69.60[b]	< 0.001
THI[†]	67.24[c]	69.34	71.96	73.80	76.32	78.14	80.20	81.59[a]	82.87	80.21	77.88	76.96	71.43[b]	< 0.001

*Different letters at 06:00 am (before), 13:00 pm (during), and 18:00 (after solar radiation) represent $P \leq 0.05$

[†] THI was class as alert condition to dairy goats from 12:00 to 15:00 pm

During the in vivo study, all data were performed at the 06:00 am (before), 13:00 pm (during), and 18:00 pm h (and after exposure to solar radiation). Afterwards, experimental goats came back to the covered pens described above, with free access to shade, feed, water, and mineral salt. In this experiment, the goats were their own control during the thermoneutral period of the day (at 6:00 h). During the exposition of heat stress, environmental data were checked continuously and physiological responses of goats were constantly observed. These care ensured that goats health conditions in face to experimental heat stress do not overpass the threshold of life risk. In the in vitro experiment, blood tubes were submitted to one of two treatments in different water baths: one at 38 °C (control treatment) and another at 40 °C (acute heat stress treatment) for 3 h. In both studies, the blood leukocytes (peripheral blood mononuclear cells (PBMCs)) were used as a cellular model because these cells have been used in several studies to evaluate heat stress responsiveness (Guerriero and Raynes 1990; Dangi et al. 2012; Kishore et al. 2013).

Data collection and sampling

The respiratory frequency (RF) and rectal (RT), dorsal (DT), tail (TT), ocular (OT), and mammary gland (MT) temperatures were measured during the 3 days of solar exposure at 06:00 am, 13:00 pm, and 18:00 pm h. RF was determined with a stethoscope placed on the rib spaces to obtain the breath movements per minute (bpm). RT was measured with a calibrated clinical thermometer with a precision of 0.10 °C. DT and TT were measured by an infrared thermometer with a precision of ± 2 °C (Incoterm, MultiTemp portable, Porto Alegre, Rio Grande do Sul, Brazil). OT and MT were measured by infrared thermography (Testo® 875-2i camera, Melbourne, Victoria, Australia), with an emissivity of 0.98, accuracy of ± 2 °C, and thermal sensibility < 50 mK. The images obtained were analyzed using IRSoft software (version 4.0), taking into account the analyses performed in other studies (Weschenfelder et al. 2013; Alejandro et al. 2014; Sathiyabarathi et al. 2016). The OT was drawn from a circle on the eye, limited by the medial and lateral commissures, and the MT was obtained by drawing a rectangle at the central part of both glands, limited by the legs (Fig. 1).

Just after the physiological measurements were performed, blood was sampled at 06:00 am, 13:00 pm, and 18:00 pm from each animal by jugular venipuncture in heparin and EDTA tubes for hormone assay and gene expression studies, respectively. Blood with EDTA was centrifuged at 1500g for 20 min at 4 °C and the leukocyte layer was transferred to a microtube and 1 ml of hemolysis solution (300 ml of ultrapure water, 54.8 g of sucralose, 6 ml of Tris-HCl, 2.5 ml of $MgCl_2$, and 5 ml of Triton 1%) was added to the leukocyte layer. Afterward, the leukocyte layer with hemolysis solution was

Fig. 1 Scheme used to obtain the superficial temperature of the ocular region and mammary gland. The mean of the ocular temperature was calculated by IRsoft 3.6 software using 1 cm of the radium; the mean mammary temperature was calculated using the middle of the mammary gland as a reference, taking a rectangle of 8 × 6 cm
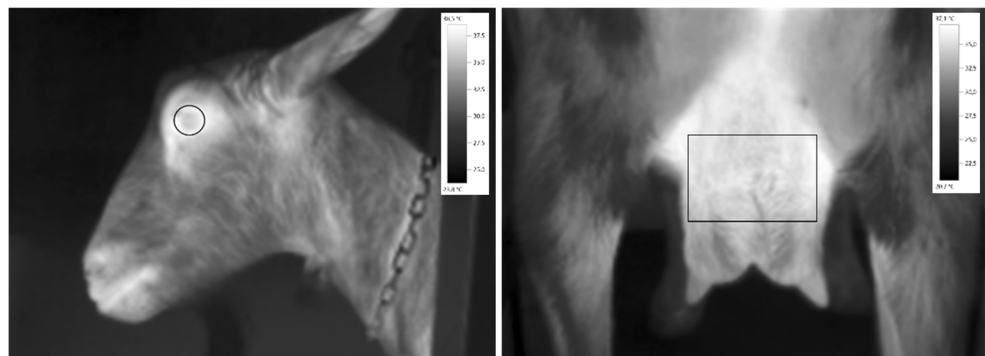
centrifuged at 12,000*g* for 2 min at 24 °C. The supernatant was discharged by inversion and the pellet containing the PBMCs was maintained at −80 °C until the RNA extraction.

Blood with heparin also was centrifuged at 1500*g* for 20 min at 4 °C and the plasma was stored at −20 °C until hormone analysis. Cort, T4, and T3 were measured using immunoassay kits (Monobind Inc., Lake Forest, CA, USA, catalog numbers 365-300, 225-300, 125-300 respectively) with sensitivities of 0.25 μg/dl, 0.04 ng/ml, and 0.04 μg/dl, respectively. The Cort intra- and inter-assay coefficients of variation were 2.8% and 6.5%, respectively. The T4 intra- and inter-assay coefficients of variation were 2.3% and 3.5%, respectively. The T3 intra- and inter-assay coefficients of variation were 3.6% and 4.1%, respectively.

The total RNA was extracted and purified using a PureLink RNA Mini Kit (Invitrogen, Carlsbad, CA, USA). The RNA concentrations were determined by Qubit® 2.0 Fluorometric Quantification (Thermo Fisher Scientific, Wilmington, DE, USA). The total RNA obtained was treated with RNase-Free DNase (Promega, Madison, WI, USA) to exclude genomic DNA contamination from the analysis. The RNA was reverse-transcribed into cDNA using a GoScript ™ Reverse Transcriptase kit (Promega, Madison, WI, USA). The qPCR analyses were performed using a PCR-RT machine (Invitrogen, Carlsbad, CA) with SYBR® Green (Invitrogen, Carlsbad, CA, USA) as the fluorescent label. Each qPCR reaction had a total volume of 20 μl and was performed using 96-well plates (Invitrogen, Carlsbad, CA, USA) and transparent adhesive tapes.

The reaction mixture was comprised of 1 μl of cDNA (mean concentration of 10 ng/μl), 10 μl of SYBR® Green Mix, 0.8 μl of the primer pair (final concentration 0.4 μM), and 8.2 μl of ultrapure water. A control reaction was included in each assay to detect contamination. The primers used in this study were for HSP60, HSP70, HSP90, Bax, Bcl-2, p53, and GAPDH (as a housekeeping gene) and all were sequenced (Table 2). The qPCR thermocycling conditions for all genes were the incubation stage at 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min, and finished with a dissociation curve. For each set of primers, the PCR efficiency was close to 100%, all primer pairs used were confirmed for their PCR efficiency, and specific products were checked by melt curve analysis and 1.5% agarose gel electrophoresis. Relative quantification of target genes was done using the Livak and Schmittgen (2001) method by comparing the expression level of the target gene with the reference gene (GAPDH).

Statistical analyses

The data were analyzed using the Statistical Analysis System (SAS 2008). The normality of the data was confirmed using the Shapiro-Wilk test. Afterwards, the body temperatures and hormonal release data were subjected to an analysis of variance by the MIXED procedure of SAS. In the model, the treatment effect (exposition to solar radiation) was considered fixed and the effects of the day, time of sampling (06:00 am before, 13:00 pm during, and 18:00 pm after solar radiation),

Table 2 Mean ± standard error of mean of rectal temperature (RT), dorsal temperature (DT), tail temperature (TT), ocular temperature (OT), mammary temperature (MT), respiratory frequency (RF), cortisol (Cort), thyroxine (T4), triiodothyronine (T3), and the relative expression of HSP60, HSP70, HSP90, Bax, Bcl-2, p53, and Bcl-2/Bax genes measured during the in vivo experiment

		Hours			
	Item	06:00 am	13:00 pm	18:00 pm	*P* value
Animal	RT (°C)	38.29 ± 0.07^c	39.63 ± 0.14^a	38.95 ± 0.05^b	0.001
	DT (°C)	24.92 ± 0.29^c	39.76 ± 0.89^a	29.48 ± 0.89^b	0.001
	TT (°C)	35.62 ± 0.42^b	38.88 ± 0.38^a	36.63 ± 0.30^b	0.001
	OT (°C)	34.77 ± 0.17^c	38.40 ± 0.30^a	36.58 ± 0.15^b	0.001
	MT (°C)	33.96 ± 0.22^c	38.73 ± 0.46^a	36.42 ± 0.30^b	0.001
	RF (°C)	49.47 ± 4.31^c	182.4 ± 11.1^a	81.87 ± 7.75^b	0.001
Plasma	Cort (ng/ml)	8.33 ± 0.74^b	12.47 ± 1.50^a	8.84 ± 0.74^b	0.001
	T4 (ng/dl)	5.10 ± 0.16^a	5.04 ± 0.11^b	5.43 ± 0.16^a	0.05
	T3 (ng/dl)	1.34 ± 0.04^{ab}	1.30 ± 0.03^b	1.45 ± 0.04^a	0.05
Leukocytes	HSP60	1.01 ± 0.23^b	2.07 ± 0.21^a	0.8 ± 0.37^b	0.004
	HSP70	0.33 ± 0.12^b	0.84 ± 0.12^a	0.87 ± 0.12^a	0.01
	HSP90	0.60 ± 0.08^b	1.14 ± 0.17^a	0.54 ± 0.07^b	0.03
	Bax	0.77 ± 0.06^a	0.60 ± 0.06^a	0.63 ± 0.06^a	0.11
	Bcl-2	1.01 ± 0.54^a	2.33 ± 0.62^a	0.44 ± 0.76^a	0.15
	p53	1.00 ± 0.24^a	0.82 ± 0.25^a	0.55 ± 0.28^a	0.52
	Bcl-2/Bax	1.29 ± 0.62^a	3.88 ± 1.36^a	0.69 ± 0.37^a	0.14

Different letters at 06:00 am (before), 13:00 pm (during), and 18:00 (after solar radiation) represent $P \leq 0.05$

and the animals were considered as random effects. As the 3 days of experiment were typical of spring and did not differ, the effect of the day was excluded from the model. Gene expression in vivo and in vitro were subjected to an analysis of variance by the GLM procedure of SAS, which separated the in vivo (exposition to solar radiation) and in vitro (38 or 40 °C) treatments, the time of sampling, and the animals as causes of variation. In the model, the treatment effect was considered fixed and the effects of the animals were considered random. Several errors of covariance structures were tested, and the one that best fit the data according to the Bayesian information criterion was selected. Pearson correlations were considered to estimate the relations between body temperatures, physiological and cellular responses, and weather data. When there was a significant effect, the means were compared using Fisher's test. Significance was defined as $P \leq 0.05$.

Results

The THI measured from 12:00 to 15:00 was classified as the alert condition because to the THI was greater than 80, at that point and BGT was superior at 40 °C, indicating the intensity of solar radiation at that time. Consequently, during the in vivo experiment, there was a significant effect of sampling time on RT, DT, TT, OT, MT, RF, Cort, T4, and T3 concentrations and HSP60, HSP70, and HSP90 expression levels when comparing the means obtained before (06:00 h), during (13:00 h), and after (18:00 h) the solar radiation. However, there was no effect of sampling time on the expression of Bax, Bcl-2, or p53 genes, nor on the relation between the Bcl-2 and Bax genes (Table 2).

In fact, the increased body temperatures, RF, Cort concentration, HSP60 and HSP90 expressions of the goats were significantly influenced by solar radiation exposure, with higher values at 13:00 than those measured at 06:00 and 18:00 (Table 2). Thus, HSP70 expression was lower at 06:00 than at 13:00 and 18:00 h. In contrast, there was a reduced T4 concentration at 13:00 compared to that at 06:00 and 18:00 h (Table 2).

In the same way, there were significant correlations between AT, BGT, RH, THI and body temperatures, physiological responses, and cellular responses (Table 3). Notably, RT had a significant and positive correlation with MT ($r = 0.93$; $P < 0.001$), OT ($r = 0.92$; $P < 0.0001$), DT ($r = 0.94$; $P < 0.0001$), and TT ($r = 0.87$; $P < 0.0001$). Thus, there were significant correlations between RT and Cort ($r = 0.67$; $P < 0.0001$), BGT and Cort ($r = 0.70$; $P < 0.0001$), and BGT and T4 ($r = -0.50$, $P = 0.007$). Furthermore, there were significant correlations between MT and T4 ($r = -0.41$; $P = 0.03$), MT and Cort ($r = 0.74$; $P = 0.004$), and MT and HSP60 ($r = 0.68$; $P = 0.001$).

In the in vivo experiment, there was a significant effect of the treatment on the expression of the target genes. In fact, when compared to the 38 °C treatment (control), leukocytes maintained in vitro at 40 °C (heat stress) showed increased expression of the HSP60, HSP70, and HSP90 genes. In the same way, the 40 °C treatment increased the expression of p53 (pro-apoptotic protein), Bcl-2 (anti-apoptotic protein), and the relationship between the Bcl-2 and Bax genes due to higher Bcl-2 expression and the maintenance of Bax expression. In this case, the HSP70 gene was overexpressed when compared to the other genes studied (Fig. 2).

Discussion

In the present study, there was significant influence of solar radiation on BGT, AT, RH, and THI; however, it is necessary to consider the heating during the day and the cooling during the night for the analysis of the environmental conditions that animals are exposed to in order to understand the physiological responses to heat stress (West et al. 2003). In the present study, the unfavorable AT was observed from 12:00 to 15:00 pm because during this period the AT was above the goats' maximum comfortable temperature, which was previously reported as between 20 and 30 °C (Brasil et al. 2000; Pereira et al. 2011; Ribeiro et al. 2016). In contrast, RH was low between 12:00 and 15:00 h when compared to the other hours of the day. Although AT and RH show different behaviors between 12:00 and 15:00 h, the THI was higher than 80 during the same period, indicating an alert condition for the goats (Silanikove and Koluman 2015).

In fact, our results confirm that both AT and RH are related to the effectiveness of the RT, DT, TT, OT, MT, and RF responses of experimental goats to deal with the subtropical climate, and necessary to return of experimental goats to homeostasis. Therefore, the mean RF values of 49.47 ± 4.32, 182.4 ± 11.1, and 81.87 ± 7.75 bpm at 06:00, 13:00, and 18:00 h, respectively, show that the experimental goats were in low, severe, and high heat stress, respectively (Silanikove 2000). In fact, solar radiation at 13:00 h increased the RF of the experimental goats and this physiological mechanism was induced by environmental heat change, in accordance with other studies (Brasil et al. 2000; Ribeiro et al. 2016). However, at 18:00 pm, the RF remained high, showing the limit of evaporative mechanisms to dissipate heat when AT is moderate but RH is high (Maia et al. 2016).

The RT in the present study was significantly higher at 13:00 h (39.6 °C) than at the other times. However, the increase of RF and the climate variations reduced RT from 13:00 to 18:00 pm. Indeed, the relation between RT, RF, and the climatic profile during the day and night permitted the experimental goats to have a lower RT at 06:00. The RT means observed in the present study were considered as

Table 3 Pearson's correlation coefficients among weather values, physiological responses, and cellular responses measured during the in vivo experiment

	RH	AT	BGT	THI	RT	TT	DT	OT	MT	RF	T3	T4	Cort	HSP60	HSP70	HSP90	Bax	Bcl-2	Bcl-2/Bax
AT	−0.95 0.001																		
BGT	−0.98 0.001	0.98 0.001																	
THI	−0.92 0.001	0.99 0.001	0.97 0.001																
RT	−0.89 0.001	0.94 0.001	0.91 0.001	0.94 0.001															
TT	−0.81 0.001	0.84 0.001	0.80 0.001	0.84 0.001	0.87 0.001														
DT	−0.94 0.001	0.98 0.001	0.97 0.001	0.97 0.001	0.94 0.001	0.84 0.001													
OT	−0.91 0.001	0.95 0.001	0.93 0.001	0.94 0.001	0.92 0.001	0.86 0.001	0.96 0.001												
MT	−0.93 0.001	0.97 0.001	0.95 0.001	0.96 0.001	0.93 0.001	0.89 0.001	0.96 0.001	0.95 0.001											
RF	−0.88 0.001	0.87 0.001	0.87 0.001	0.85 0.001	0.84 0.001	0.77 0.001	0.88 0.001	0.87 0.001	0.86 0.00										
T3	0.15 0.44	−0.01 0.96	−0.13 0.53	0.03 0.859	−0.05 0.78	−0.14 0.46	−0.03 0.87	−0.02 0.91	−0.05 0.79	−0.11 0.56									
T4	0.45 0.01	−0.45 0.01	−0.50 0.007	−0.44 0.01	−0.58 0.003	−0.26 0.17	−0.45 0.01	−0.45 0.01	−0.42 0.02	−0.44 0.01	0.23 0.21								
Cort	−0.71 0.001	0.69 0.001	0.70 0.001	0.67 0.001	0.65 0.001	0.74 0.001	0.72 0.001	0.69 0.00	0.74 0.001	0.66 0.00	−0.14 0.46	−0.28 0.13							
HSP60	−0.44 0.04	0.50 0.02	0.52 0.02	0.52 0.01	0.42 0.05	0.32 0.16	0.46 0.03	0.49 0.02	0.68 0.001	0.38 0.08	−0.05 0.80	−0.40 0.06	0.24 0.31						
HSP70	−0.01 0.94	0.02 0.96	0.02 0.90	0.01 0.96	0.08 0.66	0.02 0.95	0.07 0.69	0.02 0.89	−0.03 0.87	0.03 0.86	−0.03 0.86	0.06 0.80	0.02 0.91	0.55 0.01					
HSP90	−0.31 0.08	0.25 0.187	0.28 0.15	0.22 0.27	0.34 0.06	0.33 0.07	0.33 0.06	0.31 0.09	0.26 0.18	0.28 0.12	−0.28 0.13	−0.14 0.44	0.53 0.003	0.45 0.04	0.47 0.01				
Bax	0.37 0.05	−0.47 0.02	−0.42 0.03	−0.49 0.01	−0.35 0.06	−0.37 0.06	−0.43 0.02	−0.45 0.01	−0.55 0.004	−0.38 0.04	−0.07 0.72	0.12 0.54	−0.32 0.09	−0.08 0.73	0.62 0.001	0.18 0.34			
Bcl-2	−0.42 0.08	0.38 0.12	0.46 0.06	0.37 0.13	0.39 0.10	0.45 0.07	0.46 0.05	0.48 0.04	0.46 0.06	0.49 0.03	−0.36 0.14	−0.46 0.05	0.58 0.01	0.67 0.004	0.64 0.004	0.89 0.001	−0.36 0.15		
Bcl-2/Bax	−0.46 0.06	0.43 0.09	0.51 0.04	0.42 0.09	0.38 0.12	0.49 0.05	0.53 0.02	0.52 0.03	0.48 0.05	0.59 0.01	−0.24 0.35	−0.32 0.20	0.56 0.02	0.54 0.03	0.51 0.03	0.74 0.001	−0.53 0.03	0.92 0.001	
p53	0.005 0.98	−0.08 0.70	0.001 0.99	−0.10 0.63	−0.07 0.86	0.06 0.76	−0.04 0.83	0.03 0.88	−0.07 0.75	−0.10 0.62	−0.31 0.13	−0.04 0.82	0.067 0.75	0.29 0.21	0.27 0.18	0.46 0.01	0.37 0.06	0.77 0.001	0.63 0.01

Fig. 2 Mean ± standard error of mean of the relative expression of HSP60, HSP70, HSP90, Bax, Bcl-2, p53, and Bcl-2/Bax genes on blood leukocytes subjected to 38 °C or 40 °C. Different letters on columns represent $P \leq 0.05$
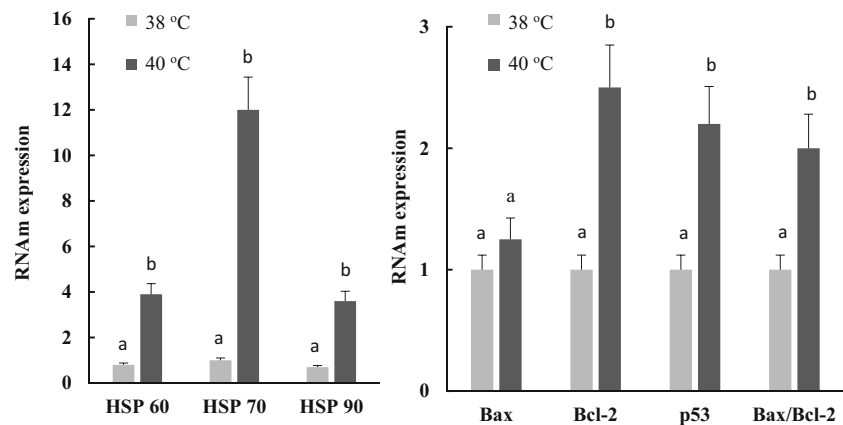
normal for the goats (Brasil et al. 2000; Ribeiro et al. 2016), suggesting that experimental goats effectively maintained their RT, though RT is a good metric to understand the animal's adaptability to heat stress (Silanikove 2000; Pereira et al. 2011). However in the present study, the solar radiation increased the RT of Saanen goats by 1.34 °C, showing that the solar radiation we observed was similar to that of other studies concerning heat stress (Brasil et al. 2000; Hamzaoui et al. 2013; Al-Samawi et al. 2014; Ribeiro et al. 2016). As these last studies showed increases from 1.2 to 1.57 °C in RT, it is possible to assume that solar radiation caused acute heat stress in the experimental goats.

The different body temperature measurements are important to understand the welfare and adaptability of animals to stressful environments (Silanikove 2000; Pereira et al. 2011). In this context, RT is a gold standard; however, other superficial temperatures, including DT, TT, OT, and MT, are important to study the effect of solar radiation; furthermore, these other body temperatures were all high and positively correlated with RT. Considering the importance of the mammary gland to a dairy animal, it is interesting to study the effect of heat stress on MT. Furthermore, the difference between these measures allows us to understand the dynamics of heat loss (Berman 2003). In this context, infrared thermography is a non-invasive tool that allows us to study superficial temperatures (Kotrba et al. 2007; Stewart et al. 2008; Stubsjøen et al. 2009; Martello et al. 2016). In the present study, DT measured before, during, and after were significantly influenced by the effect of solar exposition, and DT was more strongly correlated with RT than the other superficial temperatures studied. At the same time, in the present study, there were significant and high correlations between RT and MT and between MT and RF, Cort, T4, Bax, and Bcl-2. Consequently, the use of infrared thermography seems an interesting alternative to study the dynamics of heat loss during lactation; however, further studies are necessary to understand these relations.

In the present study, the heat stress induced by solar radiation caused a significant increase of Cort and a significant reduction decrease of T4 at 13:00 h; other authors have observed similar

effects on Cort and T4 release due to heat stress (Al-Samawi et al. 2014; Ribeiro et al. 2016). Therefore, in vivo, the heat stress significantly changed the expression of HSP60, HSP70, and HSP90 on blood leukocytes. The expression of HSP60 and 70 during the period of higher solar radiation can be explained by a cellular mechanism that maintains the conformation of other proteins on the cell, preventing their denaturation and aggregation (Deshaies et al. 1988; Sonna et al. 2002). Other authors that worked with goats in different seasons also observed a significant effect of heat stress on HSP90, HSP70, and HSP60 (Dangi et al. 2012, 2014). In this context, it is possible to argue that different HSPs are expressed to protect the cells during the different heat challenges.

Comparing the in vitro 38 and 40 °C treatments, there was a very significant difference in the expression of the target genes. In the present study, when leukocytes were maintained in vitro at 40 °C for 3 h, we observed a significant expression of the HSP60, 70, and 90 genes. Indeed, the highest overexpression in the in vitro experiment was measured for the HSP70 gene; several authors have suggested that HSP70 is the most important HSP to study in the field of heat stress (Zulkifli et al. 2010; Mishra et al. 2011; Deb et al. 2013; Romero et al. 2013; Mohanarao et al. 2014).

In vivo, there was no effect of solar radiation exposure on the expression of the Bax, Bcl-2, or p53 genes, nor on the relation between the Bcl-2 and Bax genes. However, the solar radiation influenced the expression of HSP60 and 70 genes, suggesting that these genes had a protective function during heat stress. In another way, the blood leukocytes exposed to 40 °C in vitro increased the expression of p53 (pro-apoptotic protein), Bcl-2 (anti-apoptotic protein), and the relation between the Bcl-2 and Bax genes due to higher Bcl-2 expression and maintenance of Bax expression. Similar results concerning the p53 gene and other apoptotic proteins were observed by other authors that worked with heat stress (Chen and Chuang 1999; Gu et al. 2014). In contrast to our results, other authors (Du et al. 2008) reported that mammary cells submitted to 40 °C for 3 h had decreased Bcl-2 expression and increased Bax expression. It is important to note that

different cell models were used in previous studies and the effect of heat stress on different cells can explain this contrast.

Finally, in vivo, the solar radiation exposure caused acute heat stress, punctual Cort release, and expression of HSP60 and 70 in dry Saanen goats, changing their physiological, hormonal, and cellular responses. However, the increased RF and decreased T4 and T3 were related to the homeothermy of the experimental goats. In vitro, the 40 °C treatment caused a significant increase in the expression of the p53 (pro-apoptotic protein) and Bcl-2 (anti-apoptotic protein) genes and an overexpression of the HSP60, 70, and 90 genes, suggesting that the HSP genes have protective functions. However, further studies are necessary to understand the physiological and cellular responses to heat stress.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All procedures performed in studies involving animals were in accordance with the current Federal Law n° 11.794, sanctioned by the President of the Republic on November 8, 2008. The law is available in full at http://planalto.gov.br/ccivil_03/_Ato2007-2010/2008/Lei/L11794.htm and defines the production, maintenance, and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), and regulates the scientific use of animals in Brazil. All procedures of the present study are in accordance with the National Council for Control of Animal Experimentation (CONCEA), and was approved by the Ethic Committee on Animal Use of the University of Sao Paulo, which accredited the following protocol number, CEUA 3709280316, under the responsibility of the authors.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. The animal protocol used in the experiment had the prior approval of the ethics Faculty committee, receiving the protocol number n°37090280316.

References

Alejandro M, Romero G, Sabater JM, Díaz JR (2014) Infrared thermography as a tool to determine teat tissue changes caused by machine milking in Murciano-Granadina goats. Livest Sci 160:178–185. https://doi.org/10.1016/j.livsci.2013.11.029

Al-Samawi KA, Al-Hassan MJ, Swelum AA (2014) Thermoregulation of female Aardi goats exposed to environmental heat stress in Saudi Arabia. Indian J Anim Res 48:344–349. https://doi.org/10.5958/0976-0555.2014.00453.1

Berman A (2003) Effects of body surface area estimates on predicted energy requirements and heat stress. J Dairy Sci 86:3605–3610. https://doi.org/10.3168/jds.S0022-0302(03)73966-6

Bernabucci U, Lacetera N, Baumgard LH, Rhoads RP, Ronchi B, Nardone A (2010) Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal 4:1167–1183. https://doi.org/10.1017/S175173111000090X

Brasil LHA, Wecheschler FS, Baccari Júnior F et al (2000) Efeitos do estresse térmico sobre a produção, composição química do leite e respostas termorreguladoras de cabras da raça alpina. Rev Bras Zootec 29:1632–1641. https://doi.org/10.1590/S1516-35982000000600006

Chen RW, Chuang DM (1999) Long term lithium treatment suppresses p53 and Bax expression but increases Bcl-2 expression. A prominent role in neuroprotection against excitotoxicity. J Biol Chem 274:6039–6042. https://doi.org/10.1074/JBC.274.10.6039

Dangi SS, Gupta M, Maurya D, Yadav VP, Panda RP, Singh G, Mohan NH, Bhure SK, Das BC, Bag S, Mahapatra R, Taru Sharma G, Sarkar M (2012) Expression profile of HSP genes during different seasons in goats (Capra hircus). Trop Anim Health Prod 44:1905–1912. https://doi.org/10.1007/s11250-012-0155-8

Dangi SS, Gupta M, Nagar V, Yadav VP, Dangi SK, Shankar O, Chouhan VS, Kumar P, Singh G, Sarkar M (2014) Impact of short-term heat stress on physiological responses and expression profile of HSPs in Barbari goats. Int J Biometeorol 58:2085–2093. https://doi.org/10.1007/s00484-014-0809-5

de Silva GA, De Souza BB, Alfaro CEP et al (2006) Efeito da época do ano e período do dia sobre os parâmetros fisiológicos de reprodutores caprinos no semi-árido paraibano. Rev Bras Eng Agríc Ambient 10:903–909. https://doi.org/10.1590/S1415-43662006000400018

Deb R, Sajjanar B, Singh U, Kumar S, Singh R, Sengar G, Sharma A (2013) Effect of heat stress on the expression profile of Hsp90 among Sahiwal (Bos indicus) and Frieswal (Bos indicus × Bos taurus) breed of cattle: a comparative study. Gene 536:435–440. https://doi.org/10.1016/j.gene.2013.11.086

Deshaies RJ, Koch BD, Werner-Washburne M, Craig EA, Schekman R (1988) A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides. Nature 332:800–805. https://doi.org/10.1038/332800a0

Du J, Di H-S, Guo L et al (2008) Hyperthermia causes bovine mammary epithelial cell death by a mitochondrial-induced pathway. J Therm Biol 33:37–47. https://doi.org/10.1016/j.jtherbio.2007.06.002

Gu ZT, Wang H, Li L, Liu YS, Deng XB, Huo SF, Yuan FF, Liu ZF, Tong HS, Su L (2014) Heat stress induces apoptosis through transcription-independent p53-mediated mitochondrial pathways in human umbilical vein endothelial cell. Sci Rep 4:4469. https://doi.org/10.1038/srep04469

Guerriero VJ, Raynes DA (1990) Synthesis of heat stress proteins in lymphocytes from livestock V. Guerriero, Jr and D. A. Raynes the online version of this article , along with updated information and services, is located on the world wide web at. J Anim Sci 68:2779–2783

Hamzaoui S, Salama AAK, Albanell E, Such X, Caja G (2013) Physiological responses and lactational performances of late-lactation dairy goats under heat stress conditions. J Dairy Sci 96:6355–6365. https://doi.org/10.3168/jds.2013-6665

Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. Nature 475:324–332. https://doi.org/10.1038/nature10317

Hu H, Wang J, Gao H, Li S, Zhang Y, Zheng N (2016) Heat-induced apoptosis and gene expression in bovine mammary epithelial cells. Anim Prod Sci 56:918–926. https://doi.org/10.1071/AN14420

Johnson SI, McMichael M, White G (2006) Heatstroke in small animal medicine: a clinical practice review. J Vet Emerg Crit Care 16:112–119. https://doi.org/10.1111/j.1476-4431.2006.00191.x

Kishore A, Sodhi M, Kumari P, Mohanty AK, Sadana DK, Kapila N, Khate K, Shandilya U, Kataria RS, Mukesh M (2013) Peripheral blood mononuclear cells: a potential cellular system to understand differential heat shock response across native cattle (Bos indicus), exotic cattle (Bos taurus), and riverine buffaloes (Bubalus bubalis) of India. Cell Stress Chaperones 19:613–621. https://doi.org/10.1007/s12192-013-0486-z

Kotrba R, Knížková I, Kunc P, Bartoš L (2007) Comparison between the coat temperature of the eland and dairy cattle by infrared thermography. J Therm Biol 32:355–359. https://doi.org/10.1016/j.jtherbio.2007.05.006

Lanneau D, de Thonel A, Maurel S, Didelot C, Garrido C (2007) Apoptosis versus cell differentiation: role of heat shock proteins HSP90, HSP70 and HSP27. Prion 1:53–60

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

Maia ASC, da Silva RG, Nascimento ST, Nascimento CCN, Pedroza HP, Domingos HGT (2015) Thermoregulatory responses of goats in hot environments. Int J Biometeorol 59:1025–1033. https://doi.org/10.1007/s00484-014-0916-3

Maia ASC, Nascimento ST, Nascimento CCN, Gebremedhin KG (2016) Thermal equilibrium of goats. J Therm Biol 58:43–49. https://doi.org/10.1016/j.jtherbio.2016.03.012

Martello LS, da Luz e Silva S, da Costa Gomes R, da Silva Corte RRP, Leme PR (2016) Infrared thermography as a tool to evaluate body surface temperature and its relationship with feed efficiency in Bos indicus cattle in tropical conditions. Int J Biometeorol 60:173–181. https://doi.org/10.1007/s00484-015-1015-9

McManus C, Prescott E, Paludo GR, Bianchini E, Louvandini H, Mariante AS (2009) Heat tolerance in naturalized Brazilian cattle breeds. Livest Sci 120:256–264. https://doi.org/10.1016/j.livsci.2008.07.014

McMichael AJ, Powles JW, Butler CD, Uauy R (2007) Food, livestock production, energy, climate change, and health. Lancet 370:1253–1263. https://doi.org/10.1016/S0140-6736(07)61256-2

Minton JE (1994) Function of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in models of acute stress in domestic farm animals. J Anim Sci 72:1891–1898. https://doi.org/10.2527/1994.7271891x

Mishra A, Hooda OK, Singh G, Meur SK (2011) Influence of induced heat stress on HSP70 in buffalo lymphocytes. J Anim Physiol Anim Nutr (Berl) 95:540–544. https://doi.org/10.1111/j.1439-0396.2010.01082.x

Mohanarao GJ, Mukherjee A, Banerjee D et al (2014) HSP70 family genes and HSP27 expression in response to heat and cold stress in vitro in peripheral blood mononuclear cells of goat (Capra hircus). Small Rumin Res 116:94–99. https://doi.org/10.1016/j.smallrumres.2013.10.014

Narayan E, Parisella S (2017) Influences of the stress endocrine system on the reproductive endocrine axis in sheep (Ovis aries). Ital J Anim Sci 16:640–651. https://doi.org/10.1080/1828051X.2017.1321972

Narayan E, Sawyer G, Parisella S (2018) Faecal glucocorticoid metabolites and body temperature in Australian merino ewes (Ovis aries) during summer artificial insemination (AI) program. PLoS One 13: e0191961. https://doi.org/10.1371/journal.pone.0191961

National Research Council – NRC (2007) Nutrient requirements of small ruminants: sheep, goats, cervids, and New World camelids. Washington, D. C

Ohtsubo T, Igawa H, Saito T, Matsumoto H, Park HJ, Song CW, Kano E, Saito H (2001) Acidic environment modifies heat- or radiation-induced apoptosis in human maxillary cancer cells. Int J Radiat Oncol Biol Phys 49:1391–1398

Paula-Lopes FF, Chase CC, Al-Katanani YM et al (2003) Genetic divergence in cellular resistance to heat shock in cattle: differences between breeds developed in temperate versus hot climates in responses of preimplantation embryos, reproductive tract tissues and lymphocytes to increased culture temperatures. Reproduction 125:285–294

Pereira GM, de Souza BB, de Silva AMA et al (2011) Avaliação do comportamento fisiológico de caprinos da raça saanen no semiárido paraibano. Rev Verde Agroecol e Desenvolv Sustentável 6:83–88

Pereira AMF, Titto EL, Infante P, Titto CG, Geraldo AM, Alves A, Leme TM, Baccari F Jr, Almeida JA (2014) Evaporative heat loss in Bos taurus: do different cattle breeds cope with heat stress in the same way? J Therm Biol 45:87–95. https://doi.org/10.1016/j.jtherbio.2014.08.004

Ribeiro NL, Costa RG, Pimenta Filho EC, Ribeiro MN, Crovetti A, Saraiva EP, Bozzi R (2016) Adaptive profile of Garfagnina goat breed assessed through physiological, haematological, biochemical and hormonal parameters. Small Rumin Res 144:236–241. https://doi.org/10.1016/j.smallrumres.2016.10.001

Romero RD, Montero Pardo A, Montaldo HH, Rodríguez AD, Hernández Cerón J (2013) Differences in body temperature, cell viability, and HSP-70 concentrations between Pelibuey and Suffolk sheep under heat stress. Trop Anim Health Prod 45:1691–1696. https://doi.org/10.1007/s11250-013-0416-1

SAS Institute (2008) SAS/STAT; user's guide, version 9.2. SAS Inst., Inc., Cary

Sathiyabarathi M, Jeyakumar S, Manimaran A, Jayaprakash G, Pushpadass HA, Sivaram M, Ramesha KP, Das DN, Kataktalware MA, Prakash MA, Kumar RD (2016) Infrared thermography: a potential noninvasive tool to monitor udder health status in dairy cows. Vet World 9:1075–1081. https://doi.org/10.14202/vetworld.2016.1075-1081

Silanikove N (2000) The physiological basis of adaptation in goats to harsh environments. Small Rumin Res 35:181–193. https://doi.org/10.1016/S0921-4488(99)00096-6

Silanikove N, Koluman DN (2015) Impact of climate change on the dairy industry in temperate zones: predications on the overall negative impact and on the positive role of dairy goats in adaptation to earth warming. Small Rumin Res 123:27–34. https://doi.org/10.1016/j.smallrumres.2014.11.005

Sonna LA, Fujita J, Gaffin SL, Lilly CM (2002) Invited review: effects of heat and cold stress on mammalian gene expression. J Appl Physiol 92:1725–1742. https://doi.org/10.1152/japplphysiol.01143.2001

Stewart M, Stafford KJ, Dowling SK, Schaefer AL, Webster JR (2008) Eye temperature and heart rate variability of calves disbudded with or without local anaesthetic. Physiol Behav 93:789–797. https://doi.org/10.1016/j.physbeh.2007.11.044

Stubsjøen SM, Flø AS, Moe RO, Janczak AM, Skjerve E, Valle PS, Zanella AJ (2009) Exploring non-invasive methods to assess pain in sheep. Physiol Behav 98:640–648. https://doi.org/10.1016/j.physbeh.2009.09.019

Thom EC (1959) The discomfort index. Weatherwise 12:57–59

Weschenfelder AV, Saucier L, Maldague X, Rocha LM, Schaefer AL, Faucitano L (2013) Use of infrared ocular thermography to assess physiological conditions of pigs prior to slaughter and predict pork quality variation. Meat Sci 95:616–620. https://doi.org/10.1016/j.meatsci.2013.06.003

West JW, Mullinix BG, Bernard JK (2003) Effects of hot, humid weather on milk temperature, dry matter intake, and milk yield of lactating dairy cows. J Dairy Sci 86:232–242. https://doi.org/10.3168/jds.S0022-0302(03)73602-9

Zulkifli I, Norbaiyah B, Cheah YW, Soleimani AF, Sazili AQ, Goh YM, Rajion MA (2010) A note on heat shock protein 70 expression in goats subjected to road transportation under hot, humid tropical conditions. Animal 4:973–976. https://doi.org/10.1017/S1751731110000285