Journal of Thermal Biology 84 (2019) 92–98

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Differential expression of HSF1 and HSPA6 genes and physiological responses in Angus and Simmental cattle breeds

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ARTICLE INFO

Keywords:
Adaptability
Heat stress
HSP
Taurine cattle

ABSTRACT

The aim of this study was to identify whether more and less adapted Angus and Simmental cattle differed in physiological responses and expression of the heat shock factor 1 (HSF1) and the heat shock 70 kDa protein 6 (HSPA6), when subjected to heat stress. Thirty bulls (n = 15 ANG; n = 15 SIM), extremes “more adapted" and "less adapted" within each breed were selected to the heat tolerance test. They were selected according to an index based on the average of the respiratory rate obtained on two hot summer days from one hundred bulls. Before the heat tolerance test day, animals were taken to a paddock with water, grass and shade until 7 a.m. of the following day for morning measurements. They were kept in the barn without access to water and shade until 1 p.m. for the afternoon measurements. Respiratory rate in the morning (MRR) and afternoon (ARR), hair coat surface temperature in the morning (MST) and afternoon (AST), rectal temperature in the morning (MRT) and afternoon (ART) were measured and blood samples were collected for expression analysis of the HSF1 and HSPA6 genes. The MIXED procedure of SAS was used for all statistical analysis. The more adapted Simmental group had lesser values of MRR ($P = 0.023$) and MRT ($P = 0.095$), but there was no difference within Angus breed. The ARR was greater ($P = 0.004$) in less adapted animals for both breeds. The ART was lower in the Simmental breed ($P < 0.001$). Less adapted had greater levels of mRNA of HSF1 ($P = 0.06$) and HSPA6 ($P = 0.09$). In conclusion, respiratory rate, rectal temperature and expression of the HSF1 and HSPA6 genes can be indicators of thermotolerance in taurine cattle. Both breeds show physiologically similar responses under heat stress conditions.

1. Introduction

Heat stress decreases animal production and reproductive efficiency and adversely affects livestock health (Hyder et al., 2017a). Heat stress induces a series of thermoregulatory responses that allow animals to regulate homeostasis. These responses may be physiological, such as the respiratory rate, rectal temperature, heart rate and hair coat surface temperature, or molecular, involving changes in the expression of proteins that are essential to preserve cell survival (Gupta et al., 2013).

Unlike taurine cattle (*Bos taurus*), which originate in Europe, Zebu cattle (*Bos indicus*) evolved in areas with warm climates, allowing them to acquire thermotolerance genes. Therefore, zebu breeds have a superior ability to regulate body temperature relative to taurine breeds (Hansen, 2004). One way to assess the level of adaptation of cattle undergoing thermal challenges is to measure physiological characteristics, more importantly, the respiratory rate, because its change is the

Abbreviations: Respiratory rate, RR; Morning, M; Afternoon, A; Body weight, BW; Hair coat surface temperature, ST; Temperature-humidity index, THI; Air temperature, AT; Relative humidity, RH; Angus, ANG; Simmental, SIM; Angus more adapted, ANGmore; Angus less adapted, ANGless; Simmental more adapted, SIMmore; Simmental less adapted, SIMless; Respiratory rate in the morning, MRR; Respiratory rate in the afternoon, ARR; Hair coat surface temperature in the morning, MST; Hair coat surface temperature in the afternoon, AST; Rectal temperature in the morning, MRT; Rectal temperature in the afternoon, ART

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https://doi.org/10.1016/j.jtherbio.2019.06.002
Received 27 February 2019; Received in revised form 21 May 2019; Accepted 2 June 2019
Available online 12 June 2019
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first visible sign of an animal in heat stress (Martello, 2006). Additionally, rectal temperature and hair coat surface temperature proved to be relevant characteristics in the evaluation of heat tolerance in cattle (Cardoso et al., 2015).

The stress response involves the action of heat shock proteins (HSPs). An increase in the level of these proteins in damaged cells contributes to protein repair and maintenance of cell viability because they inhibit cell death (Castro et al., 2013). HSPs are a highly conserved family of proteins that play a vital role in guiding the initial folding of proteins and subsequent refolding of partially denatured structures, conferring cell protection against stressful environments (Moura et al., 2018). Among all HSPs, HSP70 is an indicator of the amount of stress, mainly heat stress, experienced by the animal (Hyder et al., 2017b). Among other genes coding for the production of HSPs, heat shock factor 1 (HSF1) is activated during thermal stress and is mainly correlated with the induction of HSP70 gene expression (Archana et al., 2017). Among various genes of the HSP70 family, the HSPA6 gene, which encodes the HSP70 family of protein 6, was identified with a higher level of expression in goats subjected to thermal stress conditions (Banerjee et al., 2014 Mohanarao et al., 2014). This may be due to the fact that the HSPA6 gene has been developed to maintain specific vital functions under severe stress conditions (Hageman et al., 2011). In addition, this gene is almost undetectable in most tissues during normal stress-free conditions, except for certain blood cells, where it is expressed at substantial levels (Daugaard et al., 2007). Therefore, selecting for more heat-tolerant animals or breeds will be effective when suitable biomarkers can be used to select the animals and breed them to generate heat-resistant herds (Hyder et al., 2017b).

The first hypothesis is that the physiological responses for respiratory rate, hair coat surface temperature and rectal temperature will be higher in animals classified as less adapted, regardless of breed. These responses will have greater magnitude in the Angus breed than in the Simmental breed under thermal comfort but will be similar under stress. The second hypothesis is that the expression of the heat shock factor 1 (HSF1) and the heat shock 70 kDa protein 6 (HSPA6) genes will be higher in the less adapted animals and under the heat stress condition, with no difference between the breeds. The aim of this study was to identify whether Angus and Simmental cattle and, if the more and less adapted animals within these breeds, differed in physiological responses and expression of the HSF1 and HSPA6 genes, when subjected to heat stress.

2. Materials and methods

2.1. Location

All procedures were approved by the Animal Ethics Committee of the Federal University of Lavras, protocol number 018/13. The animals were from Santa Ester farm, which belongs to the *Casa Branca Agropastoril Ltda*. group, located in the region of Pouso Alegre, in the municipality of Silvianópolis, Minas Gerais (MG), Brazil (22° 01 '46 " S, 45 ° 50' 06" W, altitude of 897 m). During the heat tolerance test period, the animals were kept under semi-confinement feedlot, receiving the same diet three times a day.

2.2. Procedures

Thirty bulls (n = 15 Angus - ANG and n = 15 Simmental - SIM) were selected for the heat tolerance test. For the selection of these animals, the respiratory rate (RR) of 100 young bulls (n = 40 ANG and n = 60 SIM) was measured in the afternoon of two hot summer days. At the beginning of January 2016 (day 1) and end of January 2016 (day 2), the RR of the animals was measured by counting the number of respiratory movements in the flank region for 15 s twice, averaging the two counts and multiplying the result by four, giving the number of breaths per minute (bpm) (Silva, 2000). Animals of each breed were ranked from the lowest RR (the more adapted) to the highest RR (the less adapted), according to the mean RR over those two days. At day 1, body weight (BW) of all animals was obtained. All Angus bulls were black, and the Simmental bull breed was from a South African lineage and was red-white, which is a standard coat color for the breed. The RR in the Angus breed ranged from 39 to 95 bpm and in the Simental breed ranged from 33 to 76 bpm. More adapted animals were selected when RR values were up to 50 bpm, considered low stress, and the less adapted animals were selected with values higher than 60 bpm, considered medium high heat stress, according to Silanikove (2000).

Among the 15 Angus bulls selected, nine were classified as more adapted (ANGmore; n = 9; BW = 292.75 Kg), with a mean RR of 43.00 bpm, while six were classified as less adapted (ANGless; n = 6; BW = 237.83 Kg)with a mean RR of 71.33 bpm. The mean RR for the eight more adapted (SIMmore; n = 8; BW = 315.43 Kg)was 37.25 bpm, and that for the seven less adapted Simmental bulls (SIMless; n = 7; BW = 377.29 Kg)was 66.71 bpm. Average age of all groups was 15 months.

One day prior to the heat tolerance test, which was conducted in early March 2016, the animals were taken to a paddock adjacent to the corral with access to water, pasture and shade where they remained until 7 a.m. on the day of the test for the morning (M) measurements. The animals were kept in the corral without access to water and shade until 1 p.m., when the afternoon (A) measurements were taken. The measurements taken were respiratory rate in the morning (MRR, bpm) and afternoon (ARR, bpm), hair coat surface temperature in the morning (MST, °C) and afternoon (AST, °C) and rectal temperature in the morning (MRT, °C) and in the afternoon (ART, °C). Furthermore, blood samples were collected via jugular venipuncture into Tempus® Blood RNA (Applied Biosystems, USA) tubes to analyze the heat shock factor 1 (HSF1) and heat shock 70 kDa protein 6 (HSPA6) genes expression.

Hair coat surface temperature (ST) was measured using an infrared digital thermometer, which was pointed at the hair coat surface of the animal in the dorsal region, one hand below the spine, and the RT was recorded using digital thermometer by keeping the thermometer in contact with the rectal mucosa of animal until the thermometer beeped and displayed the temperature.

2.3. Climate conditions

On the days when the RR was measured to classify the animals according to adaptation level and on the heat tolerance test day, air temperature (AT, °C) and relative humidity (RH, %) data were collected using a Minipa® MT-242 digital thermohygrometer to obtain the temperature and humidity index (THI), developed by Thom (1958).

The AT and RH were 27.83 °C and 38.49% on Day 1, 24.80 °C and 70.11% on Day 2 and 27.00 °C and 50.03% on the Test Day (Fig. 1). This periods had average air temperature higher than 24 °C and THI around 74, which indicates heat stress condition. This type of condition is very common in hot humid summer in Brazil, which goes from December 20 to March 20, approximately.

2.4. Gene expression analysis

Total RNA was extracted from 10 ml of blood using TRIzol® reagent (Ambion, Foster City, CA, USA). The amount (ng/μl) and quality of total RNA were verified in a DeNovix DS-11 spectrophotometer (DeNovix, Wilmington, DE, USA) using Maxima SYBR Green® PCR Master Mix (Fermentas, Waltham, MA, USA) according to the manufacturer's instructions. The purity of the RNA was verified by the ratio of the absorbance values at λ_{260} and λ_{280} and was > 1.8. The RNA integrity was verified after 1.0% agarose gel electrophoresis, observing the two bands corresponding to 28S and 18S.

Genomic DNA contamination was removed from RNA by adding 1.5 μl of 10 × DNAse I (Invitrogen, Carlsbad, CA, USA) according to the

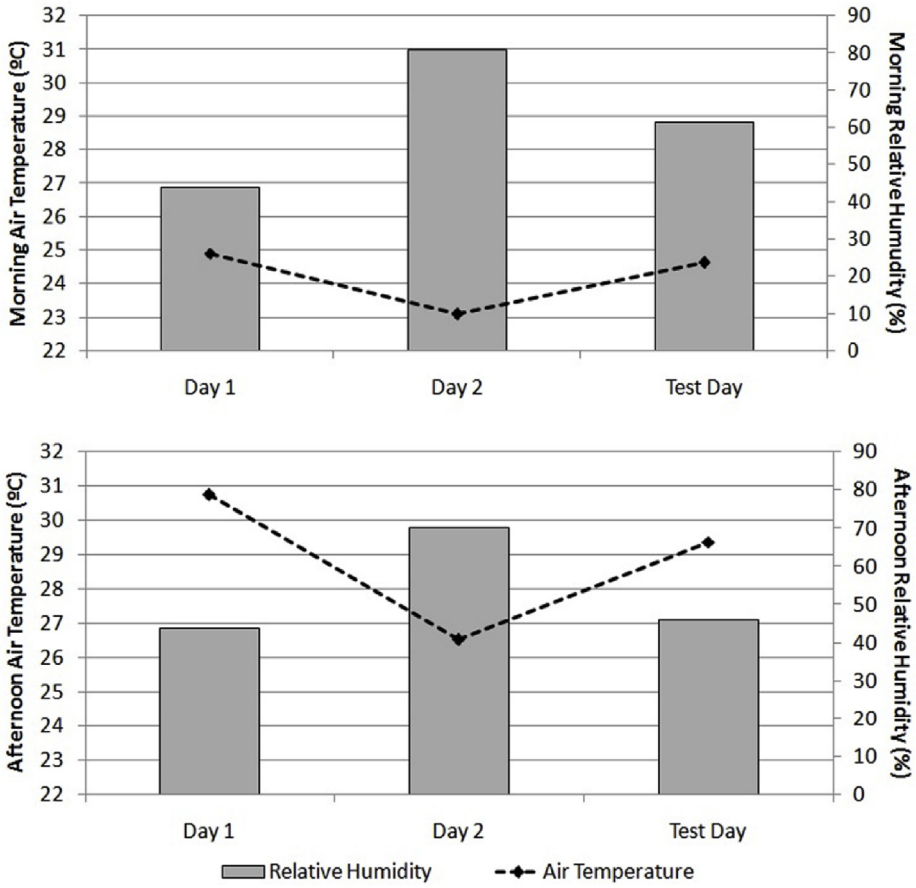

Fig. 1. Air temperature (AT, ˚C) and relative humidity (RH, %) for each day of the study. Day 1: (AT = 27.83 ˚C, RH = 38.49%) beginning of January; Day 2: (AT = 24.80 ˚C, RH = 70.11%) end of January and Test Day: (AT = 27.00 ˚C, RH = 50.03%) heat tolerance test day.

manufacturer's instructions. The samples were stored in a freezer at −80 ˚C until cDNA (complementary DNA) synthesis, which was performed using the Superscript® III First-Strand Synthesis SuperMix Kit (Invitrogen) according to the manufacturer's instructions.

Sequences for the target genes, heat shock factor 1 (HSF1) and heat shock 70 kDa protein 6 (HSPA6), as well as for the reference gene Ribosomal Protein L19 (RPL19), were obtained from the National Biotechnology Information Center (NCBI) public database. The forward and reverse primers of the three genes were designed using primer 3 plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). Table 1 shows the primer sequences for the genes used, as well as the amplicon size (bp) and annealing temperature (˚C). Each primer was designed to be complementary to two adjacent exons to prevent DNA amplification.

Gene expression analysis was performed by reverse transcription, followed by real-time quantitative reverse transcription-polymerase chain reaction (RT-qPCR) using the SYBR Green PCR Master (Fermentas) mix with the following amplification parameters: 50 ˚C for 2 min, 95 ˚C for 10 min, 40 cycles of 95 ˚C for 15 s, 60 ˚C for 1 min, ending with 95 ˚C for 15 s. The melting curve was 95 ˚C for 15 s, 60 ˚C for 15 s and 95 ˚C for 15 s.

The measurements were carried out in duplicate. The efficiency of the amplification of the target and reference genes was obtained using LinRegPCR version 2017.1 (Ruijter et al., 2009). The relative expression of the target genes was obtained according to Pfaffl (2001).

Table 1
Description of the primers for the HSF1, HSPA6 and RLP19 genes in cattle.

Gene	Primer sequence (5´- 3´)	Fragment size (bp)	Annealing temperature (C˚)
HSF1	F - CCAGCAACAGAAAGTCGTCA	92	60
	R - GGGGGATCTTTCTCTTCACC		
HSPA6	F- GAGCAAGATGAAGGAGACGG	159	60
	R - GTGGGCTCGTTGATGATTCT		
RLP19	F - ACCTGGATGAGGAGGATGAG	92	60
	R - GTACAGGCTGTGATACATGTGG		

F = forward; R = Reverse; pb = base pairs.

2.5. Statistical analyses

The data presented in this study showed a normal distribution, as verified by the Shapiro-Wilk test. The SAS version 9.0 (SAS Inst., Inc., Cary, NC, USA) MIXED procedure was used in all statistical analyses. Data from different experiments are presented as the means ± standard error (SE). A P-value < 0.10 was considered statistically significant. A pairwise comparison of means was performed using Tukey's multiple comparison test. The following statistical model was used to estimate the effects of breed, period and level of adaptation, and their interactions:

$$Y_{ijkl} = \mu + B_i + P_j + A_k + (B \times P)_{ij} + (B \times A)_{ik} + (P \times A)_{jk} + + e_{ijkl} \text{whe-}$$
$$(B \times P \times A)_{ijk}$$

re Y_{ijkl} is the dependent variable, μ is the general population mean, B_i is the mean effect of the breeds (i = 1,...,2), P_j is the average effect of period (morning and afternoon) (j = 1,...,2), A_k is the average effect of adaptation level (more adapted and less adapted) (k = 1,...,2), and e_{ijkl} is the residual assumed to be independent and to have a normal distribution.

3. Results

3.1. Climate

A difference was found in the air temperature (AT) and relative humidity (RH) between the periods of the day and between the days of evaluation of the RR for the classification of animals and the day of the test, but the Day X Period interaction was not significant (Fig. 1). In general, AT increased from the morning (24.20 °C) to the afternoon (28.88 °C) ($P < 0.001$); by contrast, AT was higher in the morning (58.04%) than in the afternoon (49.37%) ($P = 0.0039$).

On the first day of RR evaluation at the beginning of January 2016 (day 1), the AT was higher (27.83 °C) ($P = 0.0068$) and RH was the lowest (38.49%) ($P < 0.0001$) compared with the other days. On day 2, at the end of January 2016, the AT of 24.80 °C was the lowest among all days; however, RH was the highest (70.11%). On the day of the heat tolerance test, the AT (27.00 °C) did not differ from day 1, and the RH (50.03%) was lower than that on day 2 but higher than that on day 1.

The THI was significantly different only between the periods of the day, being 70.29 in the morning and 75.63 in the afternoon ($P < 0.0001$). No difference was found in THI among day 1 (74.37), day 2 (73.72) and test day (74.52) ($P > 0.05$).

3.2. Physiological measures

On days when the animals were classified according to the RR, this characteristic decreased from 67.13 ± 2.08 bpm on day 1–42.02 ± 2.08 bpm on day 2.

The mean respiratory rate (RR), hair coat surface temperature (ST) andrectal temperature (RT) for the day of the heat tolerance test are shown in Table 2. In the evaluation of the physiological characteristics, differences were found for RRM ($P = 0.023$) and RTM ($P = 0.095$) between breeds at each level of adaptation. For both breeds, the animals classified as less adapted had higher RR (ANG = 71.00 ± 4.64 and SIM = 80.57 ± 4.30 bpm), followed by the more adapted group of the ANG breed (65.25 ± 4.02 bpm), and the lowest mean RR was obtained for the more adapted SIM (54.00 ± 4.30 bpm).

A significant difference was found in RR under the heat stress condition according to adaptation level, but not according to breed, and the means were higher in the less adapted animals (109.0 ± 4.92 bpm) than in the more adapted ones (88.1 ± 4.57 bpm).

The hair coat surface temperature showed no significant differences in the factors studied. The mean ST of 31.07 ± 0.28 °C for the Angus breed did not differ from 31.04 ± 0.28 °C for the Simmental breed. No significant differences were noted in the mean ST between the more adapted animals (31.12 ± 0.27 °C) and less adapted animals (30.98 ± 0.29 °C). The mean STs for the more adapted Angus (31.31 ± 0.36 °C) and Simmental (30.93 ± 0.39 °C) bulls were statistically the same as those of the less adapted Angus (30.83 ± 0.42 °C) and Simmental (31.14 ± 0.39 °C) bulls.

Under thermal confort, the highest rectal temperatures were similar between the less adapted ANG (39.87 ± 0.18 °C) and SIM (39.89 ± 0.16 °C) groups and more adapted ANG (39.88 ± 0.15 °C) group, and the lowest rectal temperature was obtained from the more adapted SIM group (39.33 ± 0.16 °C).

Under the heat stress condition, rectal temperature showed only differences between breeds, but not between the adaptation levels, being lower in the SIM breed (40.06 ± 0.15 °C) than in the ANG breed (40.99 ± 0.15 °C) ($P < 0.001$).

3.3. Gene expression

The relative expression of the HSF1 gene is shown in Fig. 2. Expression of the heat shock factor 1 (HSF1) gene differed between the adaptation levels ($P = 0.06$); gene expression in the less adapted animals (1.99 ± 0.37) was higher than that in the more adapted ones (0.46 ± 0.50). No significant difference was found in HSF1 expression between breeds ($P = 0.363$) or period ($P = 0.394$). Additionally, no difference was noted between the Breed × Adaptation Level interaction ($P = 0.423$), Breed × Period interaction ($P = 0.260$), Adaptation Level × Period interaction ($P = 0.957$) and triple interaction Breed × Adaptation Level × Period ($P = 0.495$).

The relative expression of the HSPA6 gene is shown in Fig. 3. Like the HSF1 gene, a significant difference was found between the adaptation levels ($P = 0.09$) for the HSPA6 gene. HSPA6 gene expression was 8.18 ± 1.98 in less adapted animals and 1.04 ± 2.71 in the more adapted animals. Gene expression between breeds ($P = 0.338$) or between periods ($P = 0.503$), and between Breed × Adaptation level ($P = 0.491$), Breed × Period ($P = 0.882$), adaptation level × Period ($P = 0.436$) and Breed × Adaptation Level × Period ($P = 0.731$) was not significantly different.

4. Discussion

Air temperature (AT)increased from the morning to the afternoon period and, conversely, the relative humidity (RH) decreased, findings that are supported by the relationship between the temperature increase and atmospheric RH decrease (Ambaum, 2010).

From day 1 to day 2, beginning and end of January, the AT decreased and the RH increased. On the test day, the AT was as high as that on day 1, but the RH was intermediate between days 1 and 2. Under low temperature conditions, the animals can perform heat exchange by radiation and convection; however, with an increasing in AT and decreasing in RH, body heat dissipation changes to evaporation (Silanikove, 2000). This finding indicates that, in the afternoon, evaporation was the predominant form of heat exchange, considering that the temperatures were higher than that in the morning period. Similarly, for day 1 and test day, high temperatures and low to moderate RH were observed.

According to the classification of the U.S. Livestock Weather Safety Index (LWSI; LCI, 1970), THI values up to 74 indicate thermal comfort, values from 74 to 79 indicate heat stress alert, values between 79 and 84 indicate a hazardous situation and values higher than 84 indicate an emergency. In this experiment, a THI of 70.29 in the morning kept the animals under thermal comfort, whereas the increase to 75.63 in the afternoon led to heat stress for the animals. Despite the change in the environmental condition, the ambient temperature may not have reached significant levels, causing changes in the physiological characteristics and gene expression.

Although no significant difference was found in THI between the

Table 2
Average Respiratory Rate, Hair Coat Surface Temperature and Rectal Temperature in the Test Day for the Breed x Adaptation groups.

	Respiratory Rate		Hair Coat Surface Temperature		Rectal Temperature	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
ANGmore	65.25 ± 4.02b	93.25 ± 6.25	31.31 ± 0.36	35.06 ± 0.30	39.88 ± 0.15a	40.80 ± 0.20
ANGless	71.00 ± 4.64a	111.67 ± 7.21	30.83 ± 0.42	34.08 ± 0.34	39.87 ± 0.18a	41.18 ± 0.23
SIMmore	54.00 ± 4.30c	82.86 ± 6.68	30.93 ± 0.39	34.86 ± 0.32	39.33 ± 0.16b	39.90 ± 0.21
SIMless	80.57 ± 4.30a	106.29 ± 6.68	31.14 ± 0.39	34.93 ± 0.32	39.89 ± 0.16a	40.23 ± 0.21

[a,b,c]Means followed by a different letter in the column are significantly different according to Tukey test. ANGmore: Angus more adapted group; ANGless: Angus less adapted group; SIMmore: Simmental more adapted group; SIMless: Simmental less adapted group.

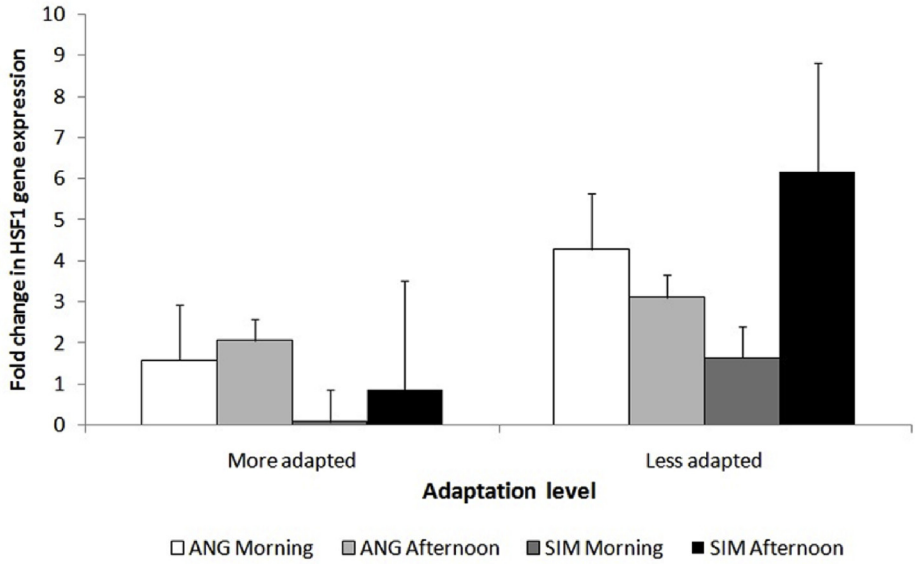

Fig. 2. Relative mRNA expression of the HSF1 gene on the day of the heat tolerance test. The vertical lines above each bar indicate +1 SE in the gene expression of the Angus (ANG) and Simmental (SIM) groups in the morning and afternoon periods.

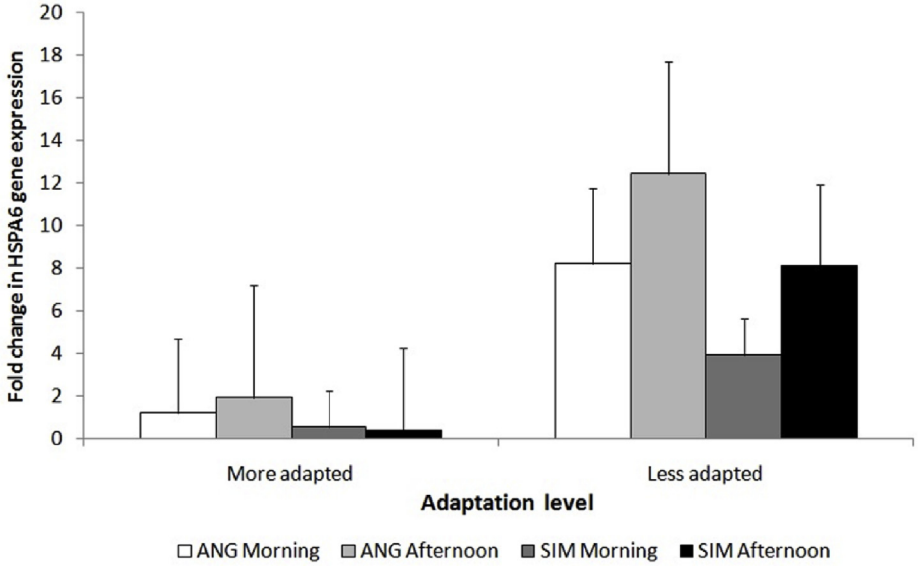

Fig. 3. Relative mRNA expression of the HSPA6 gene on the day of the heat tolerance test. The vertical lines above each bar indicate +1 SE in the gene expression of the Angus (ANG) and Simmental (SIM) groups in the morning and afternoon periods.

days, the respiratory rate (RR) decreased from day 1 to day 2, which can be explained by the temperature decreasing and the RH increasing. Climatic conditions of Day 1 caused a greater physiological response to stress through respiratory evaporative loss; however, on day 2, the predominant heat exchange was non evaporative.

The RR did not differ between the breeds but differed between the adaptation levels in both periods of the day, with higher values in less adapted animals, indicating genetic differences within each breed. A RR increase is the first physiological response to heat stress that helps in heat dissipation by evaporative cooling (Martello, 2006). Johnson et al. (2012) found that the thermo sensitive Angus cattle showed a higher respiratory rate than the thermo tolerant Romanusiano breed during summer, with air temperatures ranging from 12.67 °C to 36.2 °C and THI values from 55.42 to 91.85, a variation much larger than that observed in the present study. Changes in physiological responses such as the RR and rectal temperature vary with changes within the season to maintain normal body temperature independent of air temperature variation (Hyder et al., 2017b). A more homogeneous condition observed by the similarity of THI between the days studied in the present study allowed the breeds to behave physiologically in the same way in response to stress. Svotwa et al. (2007) identified that, in the thermoneutral condition (THI = 68.9 ± 4.2), the Simmental breed had a lower surface and rectal temperature; however, the RR was higher than those of the Brahman and Mashona breeds, which were similar in their physiological responses. According to the author, the Simmental heat tolerance was inferior to that of the other breeds. Under a thermoneutral condition, the Simmental breed may be better adapted to climatic conditions, although it is still inferior to zebu breeds; however, under heat stress, the physiological responses of the Simmental breed are similar to the British breeds, such as the Angus.

In the morning, the less adapted groups of both breeds had a higher respiratory rate, followed by the more adapted Angus group and more

adapted Simmental group with the lowest RR. This finding indicates that genetic variations may persist within these breeds, allowing adaptation to the high heat conditions of the environment, which helps to select the superior animals for reproduction.

Regardless of whether the animal is under heat stress, and despite the black coat of the Angus breed, the hair coat surface temperature did not differ for any factor studied. The mean ST for all treatments varied up to 35 °C, except for the more adapted Angus group under heat stress (35.06 °C). According to Collier et al. (2006), the temperature gradient between the core and skin is sufficiently large in cattle with a skin temperature below this value, so the animals can still use other heat exchange mechanisms effectively.

In the present study, the Angus breed had higher values than the Simmental breed for rectal temperature both under thermal comfort and heat stress conditions, which indicates genetic differences for this thermoregulatory response. In a study by Scharf et al. (2010), the rectal temperature and respiratory rate increased when the environment changed from thermal comfort (21 °C) to heat stress (26–36 °C) in Angus and Romanusiano breeds. Moreover, under both ambient conditions, Angus cattle exhibited higher values for these characteristics.

Rectal temperatures increase when they reduce evaporative heat losses, and this is due to the increase in RH (Silanikove, 2000). On Test Day, humidity showed the highest variation from the morning to the afternoon, indicating that the Angus bulls may have had more difficulty coping with the climate changes than the Simmental breed; therefore, the rectal temperature of Angus bulls was higher.

In addition to the RR, the Simmental breed also showed greater resistance. This may be due to the Simmental breed studied here belonging to a lineage of South African origin.

In a similar experiment, Ribeiro et al. (2009) observed the induction of HSF1 gene expression in Angus × Nelore (AN) crossbred animals in the afternoon in relation to the morning, with the Nelore and Senepol × Nelore crosses not showing increased expression from morning to afternoon. Senepol is an adapted and thermotolerant taurine breed capable of dissipating heat under high ambient temperatures, and the Nelore breed is a zebu breed known to exhibit high heat tolerance (Silva et al., 2018). Animals with some Angus blood, representing a breed exhibiting less adaptation to heat conditions than the other breeds, have been shown to be more sensitive to heat stress because they express more HSPs under stress conditions. This finding agrees with our study results, in which animals physiologically classified as less adapted expressed HSPs with greater intensity than the more adapted groups, regardless of breed.

In addition to the level of adaptation, one of the objectives of this study was to identify whether an effect of the time period exists on the expression of the HSF1 and HSPA6 genes. No difference was observed in gene expression for comfort or heat stress conditions. The heat stress response is triggered rapidly after acute heat stress and takes approximately 24 h for a significant change in HSP expression (Maloyan et al., 1999).

The change in time period in one day was not sufficient to observe an increase in the expression of HSPs, as demonstrated by Bharati et al. (2017), who observed constant stress for more than one day by changing the thermal comfort condition (25 ± 1 °C) of six *Bosindicus* animals to 42 °C for 23 days.

However, Ribeiro et al. (2009) identified higher expression of the HSF1 gene and a trend of HSPA6 expression with higher intensity in the afternoon under the condition of heat stress for the Angus × Nelore, Senepol × Nelore and Nelore groups. Under *in vitro* conditions, Deb et al. (2014), found higher HSP90 expression shortly after induction of heat shock and observed that mRNA expression was higher in *Bos indicus* than in crossed (*Bos indicus* x *Bos taurus*) cattle. This breed comparison results were also observed by these authors in summer conditions (37° to 42 °C). Kamwanja et al. (1994) subjected blood lymphocytes from 36 Angus, Brahman and Senepol heifers (12 from each breed) to heat shock at 42 °C for 1 h. A 2- to 3-fold increase was observed in the intracellular concentrations of HSP70, which was induced although there was no significant effect between breed × temperature. HSP70 may be indicative of chronic stress in cattle because it is correlated with higher temperatures (Gaughan et al., 2013). The environment condition from the studies cited above with experimental temperatures higher than 35 °C may have contributed to the increase in HSPs expression. In the present work, although the taurine breeds were outside their thermal comfort zone in environments with an air temperature above 25 °C (Hahn et al., 1992), the heat stress condition with an ambient temperature up to 30.75 °C in this experiment was not sufficient to induce increased expression of the HSPs *in vivo*.

Wang et al. (2017) observed increased expression of several HSP genes, including the HSPA6 gene, in the liver of taurine milk cows under summer conditions with high temperatures and THI values between 77 and 81, compared with cows under thermal comfort, with THI values between 50 and 61. Compared with this study, the THI values were lower for cows under thermal comfort, while they were similar under heat stress (Fig. 1). The expression of the HSP70 family genes varies with species, breeds, age and tissue type depending on the stress experienced by the cell, explaining the variations in thermotolerance (Hyder et al., 2017b).

One of the objectives of this study was to identify breed effects on gene expression. Banerjee et al. (2014) reported an increase in the summer expression of HSP70 family genes, such as HSPA8, HSPA1A and HSPA6, in goat breeds adapted to cold weather. Conversely, the expression increased in the winter for the breeds adapted to heat. The Angus and Simmental breeds are of European origin, animals adapted to a cold climate; therefore, an increase in the expression of the HSF1 and HSPA6 genes was expected from the period of thermal comfort to heat stress with higher temperatures, which did not occur. However, the same author reported that, due to variations in the adaptation of animals to different environmental conditions, the pattern of HSP70 gene expression is specific to breed and species.

With or without stress condition and regardless of breed, the stress response at the molecular level was similar. However, physiologically less adapted animals express a higher level of these genes, suggesting that the HSF1 and HSPA6 genes may be indicators of genetically superior animals with respect to the level of adaptation. It may also indicate that the expression of these genes is related to the metabolic pathway that regulates the RR because the less adapted animals had a higher RR and higher expression of these genes.

5. Conclusion

The Simmental breed has proven to be more resistant than the Angus breed, under thermal comfort conditions, when the ambient temperature is up to 25 °C and the Temperature and Humidity Index is less than 74. However, both breeds present physiologically similar responses under heat stress conditions.

Considering that higher expression of the HSF1 and HSPA6 genes was observed in animals classified as less adapted—that is, those with a higher respiratory rate—it is believed that these genes can be used as thermotolerance indicators in taurine cattle because they are related with more physiologically adapted animals, within each breed, to the subtropical climate conditions.

Like the genes studied, the physiological characteristics respiratory rate and rectal temperature proved to be good indicators of biotypes better adapted of cattle raised under subtropical climate conditions.

Declarations of interest

None.

Acknowledgments

Our thanks to Casa Branca Agropastoril Ltda. for providing the

animals for this study, to CAPES (Coordination and Improvement of Higher Level Personnel), Brazil and CNPq (National Council for Scientific and Technological Development), Brazil, for granting scholarships to the students of this project. This material is based upon work supported by FAPEMIG (Foundation for Research Support of the State of Minas Gerais), Brazil, under Agreement No. CVZ - APQ-00689-13.

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