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Pupillary dilation as a thermal stress indicator in boer crossbred goats maintained in a climate chamber

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ABSTRACT

Goats are homeothermic animals considered rustic from the bioclimatic point of view, but a reduction in their productive efficiency occurs under unfavorable thermal conditions. The present study evaluated pupil dilation as an indicator of thermal stress in Boer crossbred goats maintained under controlled environmental conditions. Six male crossbred goats of the Boer breed were distributed in a completely randomized design and submitted to temperatures of 26, 29 and 33 °C and 68% relative humidity. Pupillary dilation (PD), pupillary temperature (PT), respiratory frequency (FR), heart rate (HR), surface and rectal temperatures (ST and RT), body weight gain (BWG) and feed conversion (FC) were monitored. An increase in the PD of the animals was observed with elevated air temperature. Specifically, pupillary area increased by 26.96 mm^2 at 33 °C compared to 26 °C. PD is shown as an efficient response for measuring the level of thermal stress in goats.

1. Introduction

The breeding of meat goats stands out as a potential opportunity for the socioeconomic development of the Brazilian Northeast due to the high degree of adaptation of the species to regional climatic conditions. This region is characterized by high air temperatures due to the high incidence of solar radiation, which interferes negatively with the development and production of animals due to the stressful thermal conditions of the environment (de Souza et al., 2012).

Some physiological changes are relevant indicators of thermal stress in animals kept in thermally unfavorable environments. One such physiological response is pupillary area, which varies with the dilation or constriction of the iris. According to Laeng et al. (2012), pupillary area can be affected by factors such as excitement, emotion and stress. The same authors, when conducting an experiment with constant illumination, verified the occurrence of pupillary dilation when subjects were submitted to activities involving attention and behavioral decisions. This was explained by an increase in the production and release of monoamines (norepinephrine) in the mediations of the *locus coeruleus* as a result of the release of these neurotransmitters.

The pupil is known to reflect the activities of the autonomic nervous system (ANS): In particular, the pupil has been shown to expand (mydriasis) as a consequence of mental effort and exposure to stressors (Lempert et al., 2015; Pedrotti et al., 2014). Therefore, we hypothesize that there will be a substantial increase in the pupil size of animals when subjected to thermal stress.

The objectives of this study were to measure pupil dilation using digital images of Boer + no defined racial pattern (NDRP) crossbred goats exposed to different temperatures in a climatic chamber and to correlate this variable with other physiological responses and air temperature.

2. Materials and methods

2.1. General

The procedures performed in this study were approved by the Research Ethics Committee (CEP) of the Federal University of Campina Grande, Paraíba, Brazil, CEP Protocol N°. 284-2015.

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2.2. Animals and housing

Six male crossbred goats (¾ Boer breed and ¼ no defined racial pattern (NDRP)) were selected from breeding herds suitable for meat production, with an average weight of 25.06 ± 4.43 kg and mean age of six months. The animals were housed in metal stalls with dimensions of 1.15 × 0.50 × 0.84 m in length, width and height, respectively.

2.3. Experimental design

The animals were grouped using a completely randomized design, with three treatments (air temperatures) and six replicates (animals). The air temperature values used in the experiment were chosen based on the thermal comfort zone (TCZ) for goats mentioned by Souza et al. (2008). Thus, the animals were submitted to 3 different mean-controlled temperatures: T1 = 26 °C (thermal comfort zone), T2 = 29 °C (limit temperature between comfort zones and thermal stress) and T3 = 33 °C (TCZ above), with relative humidity and wind speed averages of 68% ± 4% and 1 m/s, respectively.

2.4. Experimental procedures

For each temperature, a period of five days for adaptation to the controlled environment as well as handling and feeding and a period of ten days for data collection were adopted. At the beginning of each experimental stage and between the end of each treatment and the beginning of the next, the animals were exposed to ambient air temperature and relative humidity (with an open chamber) for the restoration of their physiological functions. The duration of the full experiment was 110 days.

At each stage of study in the climatic chamber, the animals were submitted to a cycle of 8/16 h (experimentalair temperature/ambient air temperature). The chamber was turned on each day at 7 a.m., allowing 1 h to stabilize the air temperature and internal relative humidity. Following stabilization, the experimental period began at 8 a.m., with the animals remaining inside the closed chamber until 4 p.m.

Animal feed and water were provided *ad libitum*. The feed supplied to the animals consisted of Tifton hay (*Cynodon dactylon* (L) Pers), which was 43.0% of the total volume of the feed, as well as corn meal (26.6%), molasses (2.5%) soybean oil (2.0%), mineral salt (1.5%) and calcitic limestone (0.4%), according to the composition indicated by NRC (2007).

During the 8 h of daily experimentation, the illuminance inside the chamber was maintained at 257 lx to avoid light influence on the dilation or contraction of the animals' pupils.

2.5. Data collection

Images were captured while the chamber remained closed to monitor the pupillary dilation of the animals during the 10 days of each treatment. The images were captured without direct contact with the animals using a hardware structure consisting of a high-resolution camera, a specific microcontroller Arduino® UNO, a module 5 V relay, and an HC-SR04 Ultrasonic Sensor.

Ultrasonic sensors were installed in the cages (on the drinking fountains) to identify the presence of the animals in search for water. Upon identifying the presence of the animal, the sonar would send an electrical pulse to the Arduino microcontroller, which then sent electrical pulses to the USB input of the cameras, triggering the capture of pupil images of the animals.

A reference scale with 5-mm intervals was set in the horn of the animals to allow for the conversion of pupil dimensions from pixel-scale images to millimeters.

Due to the unique shape of the goat eyeball and pupil (ellipsoidal), the dimensions of the major diameter (horizontal), small diameter (vertical) and pupillary area (Fig. 1) were collected. To obtain these pupillary dimensions, 30 photos of each animal in each treatment were selected for analysis.

The pupil temperature of the animals was monitored by capture of thermograms of the eyeball using a Fluke® Ti55FT thermographic camera. Thermal images were collected every three days at 9 a.m. in each proposed treatment. The thermograms were analyzed using SmartView® software version 3.1, which obtained the average temperature of the animals' pupil in the three experimental thermal conditions adopting 0.98 emissivity.

In addition to the physiological responses of pupil dilation and air temperature, data on respiratory frequency (RF), heart rate (HR), surface temperature (ST) and rectal temperature (RT) were collected. These variables were collected every three days at each experimental air temperature and were always collected in the morning following the methodology used by Lucena et al. (2013).

Feed consumption was recorded daily and weight gain was measured by the daily mean weight gained by the animals during the ten days of each treatment. The feed conversion was calculated as the ratio between dry matter intake and the how much the animals converted to live weight.

2.6. Statistical analysis

Normality and hypothesis tests were performed using Assistat statistical software (Silva and Azevedo, 2016), where data were analyzed using analysis of variance (ANOVA) and F tests. A Tukey test was used to compare averages at a 5% ($P < 0.05$) error probability. The normality of the data was verified through the Shapiro-Wilk test. All results are presented as the mean ± (S.E.M.). In addition, the pupil area was correlated with air temperature and other physiological responses.

3. Results

3.1. Pupillary responses

The smaller and larger diameter and pupillary area of the animals significantly differed with increasing air temperature ($P < 0.05$) across the three experimental treatments (Table 1), with an increase of 26.96 mm^2, or 37%, in pupillary area between the temperatures of 26 °C (within the thermal comfort zone for goats) and 33 °C (thermal stress condition). This finding demonstrates the influence of air temperature on the pupillary reactions of the study animals.

The increase in air temperature also caused an increase in pupil surface temperature (Table 1), with a significant difference ($P < 0.05$) between 26 °C and the two other air temperatures (29 and 33 °C).

3.2. Physiological and production responses

Table 2 shows that the RF, HR and ST were significantly ($P < 0.05$) higher as the air temperature diverged from the thermal comfort zone for goats. The rectal temperature did not differ ($P < 0.05$) following the increase in air temperature from 26 to 29 °C; however, there was a significant increase ($P < 0.05$) in RT at 33 °C.

BWG was inversely proportional to the increase in air temperature, and the 26 and 29° C treatments differed significantly ($P < 0.05$) from the 33° C treatment. FC increased ($P < 0.05$) when the animals were exposed to thermal stress conditions.

Strong correlation between air temperature and pupillary dilatation was observed (Table 3), and the linear increase in pupillary area with temperature increase was supported by a Pearson correlation coefficient (r) higher than 0.999.

The dilation of the pupil also showed high positive linear correlations with other physiological responses, with correlation coefficients of r = 0.950, 0.999, 0.994 and 0.965 for RF, HR, ST and RT, respectively.