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# Metabolic and behavior responses of lactating goats under heat stress

Ahmed A.K. Salama *, Soufiane Hamzaoui, Elena Albanell, Xavier Such, Gerardo Caja

*Group of Research in Ruminants (G2R), Department of Animal and Food Sciences, Universitat Autònoma de Barcelona, 08193, Bellaterra, Barcelona, Spain*

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ABSTRACT

Heat stress (HS) negatively affects animal performance, but little is known about energetic metabolism and behavior changes in dairy goats under HS conditions. Eight multiparous Murciano-Granadina dairy goats (43.3 ± 1.6 kg BW; 2 ± 0.04 L milk/d; 81 ± 3 days of lactation) were kept in metabolism cages and randomly assigned to two treatments varying in the temperature humidity index (THI). The design was crossover (two 28-d periods), and treatments were: 1) thermal neutral (TN; 15–20C, 40–45% humidity, THI = 59–65), and 2) heat stress (HS, 12 h/d at 37°C and 40%, and 12 h/d at 30°C and 40%, THI = 86 and 77, respectively). Jugular silicon catheters were fitted, and glucose tolerance test (0.25 g/kg BW), insulin tolerance test (4.6 μg/kg BW) and epinephrine challenge (2 μg/kg BW) were conducted. Before and after each metabolite administration, blood samples were collected for the analysis of insulin, glucose, and nonesterified fatty acids (NEFA). Also, behavior variables (position changes, duration of remaining standing, as well as eating and drinking bouts and duration) were observed at day 3 of each period by video cameras. Heat stress reduced ($P < 0.01$) feed intake (−29%), milk yield (−10%), milk fat (–12%), milk protein (–14%), and milk casein (–13%). Goats in both groups had similar blood NEFA after insulin administration, but NEFA values were greater ($P < 0.05$) in TN than HS goats after epinephrine infusion. The HS goats secreted lower ($P < 0.05$) amounts of insulin than TN goats in response to the glucose tolerance test. Additionally, TN and HS goats had similar number of eating bouts, but the duration of each bout was shorter in HS than in TN. Also, HS had greater number of drinking bouts with no differences in drinking bout durations between groups. In conclusion, body lipid tissue of HS goats became more resistant to lipolysis, making them unable to mobilize body fat reserves despite the negative energy balance. In addition, the reduction in feed intake by HS was because of the shorter time of eating bouts, whereas the greater water consumption was related to the increase in drinking bouts.

## 1. Introduction

Heat stress (HS) negatively impacts productivity and health of livestock (Baumgard and Rhoads, 2013; Salama et al., 2014; Sejian et al., 2018). Adaptations to cope with HS include, but not limited to, greater sweating rate, elevated respiration rate, vasodilation with increased blood flow to the skin surface, reduced metabolic rate, decreased dry matter intake (DMI), and altered water metabolism (reviewed by Baumgard and Rhoads, 2013; Salama et al., 2016; Sejian et al., 2018). Circulating $T_3$ and $T_4$ also decline by up to 25% (Silanikove, 1992), which is consistent with the decrease in metabolic rate, DMI, and production under HS conditions.

Heat-stressed cows (Baumgard and Rhoads, 2013) and goats (Hamzaoui et al., 2020, 2021) suffer negative energy balance (EB). One typical response to negative EB is a reduction in circulating insulin coupled with a decrease in systemic insulin sensitivity (Bauman and Currie, 1980). The reduction in insulin action allows adipose tissue lipolysis and the mobilization of nonesterified fatty acids (Bauman and Currie, 1980). However, in case of heat-stressed dairy cows (Rhoads et al., 2009) and ewes (Mehaba et al., 2021), blood nonesterified fatty acids (NEFA) levels do not vary compared to thermal neutral (TN) animals despite the reduced DMI. The lack of an elevated NEFA response happens even though acute HS causes an increase in blood levels of cortisol, norepinephrine, and epinephrine, which are catabolic hormones that normally stimulate lipolysis and adipose mobilization (Collier and Gebremedhin, 2015). Hormonal challenges and glucose tolerance test have been applied in heat-stressed dairy cows (Wheelock et al., 2010) and dairy sheep (Mehaba et al., 2021) to understand the metabolic changes induced by HS. However, little is known on the response of heat-stressed dairy goats to such metabolic challenges.

Behavioral adaptive mechanisms to HS include alterations in standing and lying times as well as the frequency of drinking,

* Corresponding author.
*E-mail address:* ahmed.salama@uab.cat (A.A.K. Salama).

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**Table 1**
Ingredients, chemical composition, and nutritive value of the total mixed ration used for dairy goats.

| Item | Total mixed ration |
|---|---|
| Ingredient, % | |
| Alfalfa hay | 70.0 |
| Barley, ground | 14.4 |
| Corn, ground | 8.4 |
| Soybean hulls | 4.3 |
| Soybean meal | 2.5 |
| Molasses | 0.30 |
| Sodium bicarbonate | 0.03 |
| Salt | 0.03 |
| Dicalcium phosphate | 0.01 |
| Calcium carbonate | 0.01 |
| Vitamin-mineral complex | 0.02 |
| Component, % | |
| Dry matter | 89.3 |
| Crude protein | 17.5 |
| Ether extract | 1.93 |
| Neutral detergent fiber | 43.8 |
| Acid detergent fiber | 27.0 |
| Nutritive value[1] | |
| UFL,[2] /kg | 0.84 |
| $NE_L$, Mcal/kg | 1.47 |
| PDI,[3] g/kg | 89.5 |
| PDIA,[4] g/kg | 40.1 |
| Ca, g/kg | 8.24 |
| P, g/kg | 2.60 |

[1] Calculated according to the French National Institute for Agricultural Research (Institut National de la Recherche Agronomique (INRA, 2018).
[2] Feed units for lactation (1 UFL = 1.76 Mcal of $NE_L$).
[3] Protein digestible in the intestine from dietary and microbial origin.
[4] Protein digestible in the intestine from dietary origin.

rumination, urinating and defecating (Darcan et al., 2008; Sejian et al., 2018). Despite the numerous published studies on the impact of HS on production and reproductive variables, little is known on the detailed changes in animal behavior caused by HS, especially in dairy goats.

We hypothesized that HS would result in significant changes in energetic metabolism and behavior in dairy goats. Evaluating these alterations would help in understanding better the performance under HS conditions as well as establishing effective strategies to alleviate its negative effects. The objectives of the current study were to evaluate the response of dairy goats to glucose, insulin, and epinephrine challenges under HS conditions. Additionally, video recording was used to monitor goat behavior (eating, drinking, position changes) throughout the day under TN and HS conditions.

## 2. Materials and methods

### 2.1. Animals and management conditions

The procedures used in the current study were approved by the Ethical Committee of Animal and Human Experimentation of the Universitat Autonoma de Barcelona (Reference 09/771). Also, animal care conditions and management practices were according to the Spanish Royal Decree 53/2013 on the protection of animals used for experimental purposes, and the recommendations of the Ministry of Agriculture, Food and Environment of Spain for the welfare of livestock.

Eight multiparous Murciano-Granadina dairy goats in mid-lactation (43.3 ± 1.6 kg BW; 2 ± 0.04 L milk/day, 81 ± 3 days of lactation) were used from the herd of the experimental farm of the Universitat Autonoma of Barcelona. Goats were divided into two balanced groups (n = 4 goats/group) according to milk yield and milk composition. The experimental design was a crossover design with two periods of 28 days each, and two climatic treatments that differed in the temperature-humidity index (THI) values. The environmental conditions were thermal neutral (TN; 15–20C, 40–55% relative humidity, THI = 59–65), and heat stress (HS; 12-h day at 37°C, 40 ± 5% relative humidity, THI = 86; and 12-h night 30°C, 40 ± 5% relative humidity, THI = 77). For HS goats, the temperature was increased gradually (1 d at 25°C, 1 d at 30°C). Under both conditions, photoperiod was 12-h light (0800–2000 h) and 12-h dark (2000–0800 h). Goats had a 3-wk pre-experimental period under TN conditions for the adaptation to the diet and metabolic cages. All goats remained at TN conditions for 7 days after the first period (day 1–28), and during the second period (day 36–63), goats were switched to the opposite treatment.

Goats were in 2 adjacent rooms with identical feeding conditions and facilities. Throughout the experiment (December to March), the room temperature for TN goats was maintained using electric heaters (3.5 kW; General Electric, Barcelona, Spain) equipped with a thermostat. The room of HS goats was provided with a temperature and humidity controlling system (Carel Controls Ibérica, Barcelona, Spain). A continuous 90 $m^3$/h air turnover was maintained throughout the experiment.

Data of environmental temperature and humidity were recorded every 10 min throughout the experiment by a data logger (Opus 10, Lufft, Fellbach, Germany). The THI values were calculated according to NRC (1971).

Goats were milked once daily (0800 h) with a portable milking machine (Westfalia-separator Ibérica, Granollers, Spain) provided with recording jars (3 L ± 5%). Goats were individually fed *ad libitum* a total mixed ration formulated according to INRA (2018). Feed was offered once daily at 0900 h at 115% of expected intake. Ingredients, chemical composition, and nutritive value of the ration are shown in Table 1. Drinking water was freely available at room temperature.

### 2.2. Performance variables

Daily rectal temperatures and respiration rates were recorded at 0800, 1200, and 1700 h. The rectal temperature was measured by a digital clinical thermometer (Model ICO Technology "mini color" Barcelona, Spain). The respiration rate was measured by counting the inhalations and exhalations for 60 s with the aid of a digital chronometer (Model 900,400; Deltalab, Barcelona, Spain).

Goats were weighted on two consecutive days at the start and end of each experimental period using a digital scale (Tru-Test AG500 Digital Indicator, Auckland, New Zealand; accuracy ±20 g) to measure the change in BW. Additionally, BW values were used to calculate net energy balance using the following equation: energy balance = net energy intake – ($NE_M$ + $NE_L$).

Net energy for maintenance ($NE_M$) was calculated using the following equation: $NE_M = (0.0406 \times BW^{0.75})$ according to INRA (2018). Maintenance costs were increased by 30% for HS goats as recommended by NRC (2001). Net energy for lactation ($NE_L$) was calculated by using the following equation: $NE_L$ = milk yield × [0.389 + 0.0052 (fat, g/kg – 35) + 0.0029 × (protein, g/kg – 31)] according to INRA (2018).

Feed intake and water consumption were measured daily throughout the study. Feed samples were collected before the beginning of each experimental period and were analyzed for DM, crude protein, acid detergent fiber, and neutral detergent fiber according to AOAC International (2003).

Milk was weighed by a digital scale (Mobba industrial, Barcelona, Spain) and registered daily. Milk samples were collected twice per week for the analysis of total solids, fat, protein (N × 6.38), and casein using a NIRSystems 5000 scanning monochromator (FOSS, Hillerød, Denmark) according to Albanell et al., 2003). A milk aliquot was stored at −25°C to determine milk osmolality using a Fiske 110 osmometer (Fiske Associations, Norwood, MA).

Urine spot samples were collected between 0800 and 0930 h, 1400 and 1530 h, and 1800 and 1930 h, for 5 consecutive days during weeks 1 and 4. Urine samples (10 mL) were acidified with 1 mL of 10% $H_2SO_4$,

composted for each goat and stored at −25°C until analyses. Urine samples were diluted (1:10) and analyzed for uric acid and creatinine concentrations using commercial kits according to the manufacturer's instructions (Biolabo SA, Maizy, France). Urine concentrations of uric acid and creatinine were used to calculate uric acid index (UAI) as a proxy of microbial protein synthesis:

$$UAI = (\text{uric acid concentration} / \text{creatinine concentration}) \times BW^{0.75}$$

### 2.3. Blood variables

Blood samples (10 mL) were collected before feeding and milking at days 7, 14, 21 and 28 from the jugular vein into vacutainers (Venoject, Leuven, Belgium). Plasma was obtained by centrifugation of blood for 15 min at 1500 × *g*, and stored at –25°C for the analysis of blood urea N, glucose, NEFA, β-hydroxybutyrate (BHB), lactate, and insulin. Glucose was determined by Trinder method using Glucose GOD-PAP kit (Biolabo SA, Maizy, France). The NEFA were analyzed by the colorimetric enzymatic test ACS-ACOD method using a commercial kit (Wako Chemicals, Neuss, Germany). The BHB was determined by kinetic enzymatic method using commercial kit (RANBUT, Randox®, Crumlin, UK). Insulin was measured by ELISA using a commercial kit (Mercodia Ovine Insulin ELISA, Mercodia®; Uppsala, Sweden). Lactate was determined by enzymatic method (Olympus System Reagent®, Beckman Coulter®, Nyon, Switzerland). In addition, whole blood without anti-coagulants was collected and a single drop was immediately applied to disposable cartridges (iSTAT Crea cartridges, Abbott Point of Care Inc., Princeton, NJ, USA) for the analysis of creatinine.

### 2.4. Hormonal challenges and glucose tolerance test

Indwelling jugular silicone rubber catheters (Nutricath Silicone, 60 cm length and 14-gauge, Vygon, Valencia, Spain) were inserted on day 19 of the 2nd period. On days 22, 24, and 26 glucose tolerance test (GTT; 0.25 g/kg of BW), insulin tolerance test (4.6 μg/kg of BW), and epinephrine challenge (2 μg/kg of BW) were performed, respectively. Dextrose (D9434, Sigma-Aldrich, St. Louis, MO), insulin (bovine insulin from pancreas, I6634, Sigma-Aldrich), and epinephrine (E4250, Sigma-Aldrich) were diluted in sterile 0.9% NaCl solution (Vitulia 0.9%, Laboratorios ERN S.A., Barcelona, Spain). Solutions of glucose (500 μg/mL), insulin (230 μg/mL), and epinephrine (100 μg/mL) were sterilized by filtration through 0.22-μm polyether-sulfone filters (Millex-GP, Millipore; Merck Life Science SLU, Madrid, Spain) using 1 filter for each 200 mL of solution and kept at 4°C.

On the day of each challenge, blood samples were collected at −30, −20, −10, 0, 5, 10, 20, 30, 45, 60, 90, and 120 min relative to the administration. After each blood sampling, the catheters were flushed with heparinized 0.9% saline solution (500 IU/mL; Clexane 4000 UI, Sanofi Aventis, Paris, France). Samples were collected by syringe into glass tubes containing 250 units of sodium heparin and were immediately placed on ice. After centrifugation of whole blood for 15 min at 1500 × *g* and 4°C, plasma was divided into different aliquots and stored at −25°C for subsequent analysis of plasma insulin, glucose, and NEFA concentrations as indicated above.

The area under the curve (AUC) of metabolite responses to GTT, insulin tolerance test, and epinephrine challenge were calculated by the trapezoidal method. The pre-challenge values (i.e. − 30, −20, −10, and 0 min) were averaged and considered as the basal value of each metabolite. Values of peaks or nadirs of each metabolite after infusions were recorded. Clearance rates for GTT and insulin tolerance test were calculated according to Kerestes et al. (2009).

### 2.5. Behavior variables

Eight digital color cameras (model VCAM—420CA, Circontrol1, Barcelona, Spain) with a focal lens (model LTC 0500/50, Philips, Eindhoven, the Netherlands), fitted with infrared illuminator were used (1 camera / goat). Videos were digitized and stored on SATA (Serial Advance Technology Attachment) hard disks of 500 GB each. The videos were watched using a digital recorder (VDVR-9NX Circontrol1, Barcelona, Spain) with screen and remote control, which allowed the manual control of the video. Due to the accuracy of the recorder, the images were displayed at a standard frame rate of 25 pictures per second. The following 6 behavior indices were measured for each goat:

1) Time spent while standing: the time duration spent by the goat standing for different activities (eating, drinking, or idling).
2) Frequency of position changings: number of times the goat changes its position from standing to lying down or vice versa.
3) Eating bouts: number of times the goat visits the feeder and eats.
4) Eating duration: the duration taken by the goat eating from the feeder.
5) Drinking bouts: number of times the goat visits the water trough and drinks.
6) Drinking duration: the duration taken by the goat drinking from the water trough.

According to our previous observations (Hamzaoui et al., 2013) and the current study (see hereafter), goats suffered maximum HS during the first 3 days. Therefore, the 3rd day of each treatment (TN and HS) in each period (1 and 2) was chosen to obtain the behavior variables. The activity throughout the day was considered as diurnal (12 h day from 0800 to 2000 h) and nocturnal (12 h dark from 2000 to 0800). Data for behavior observations were recorded onto an Excel spreadsheet (Microsoft Office 2013) which enabled the calculation of the duration of each behavior variable.

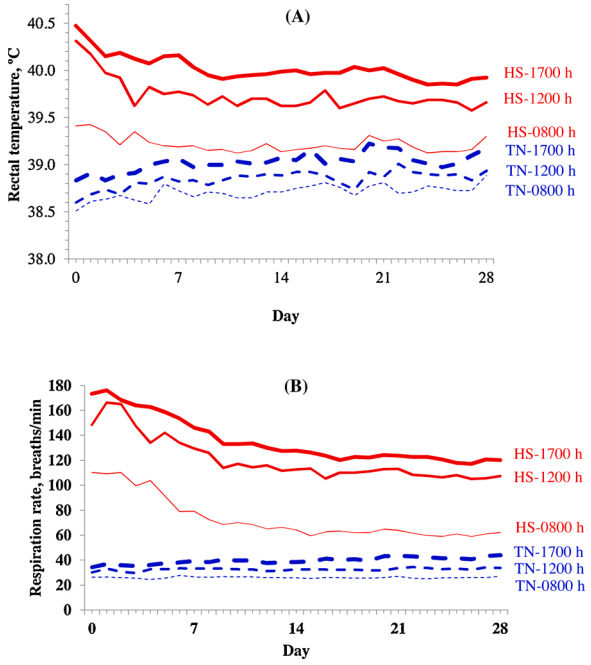


**Fig. 1.** Mean rectal temperature (A) and respiration rate (B) throughout the day (0800, 1200, and 1700 h) in dairy goats under thermal neutral (TN; blue dashed curves) and heat stress (HS; red solid curves) conditions. The SEM values were 0.07°C and 4 breaths/min for rectal temperature and respiration rate, respectively. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

**Table 2**
Performance of Murciano-Granadina dairy goats under thermal neutral (TN, n = 8) and heat stress (HS, n = 8) conditions. Values are least squares means and SE of the mean (SEM).

| Item | TN | HS | SEM | Effect ($P <$) | | |
|---|---|---|---|---|---|---|
| | | | | Treatment | Period | T × P[1] |
| Body weight change, kg | 5.30 | –1.28 | 0.72 | 0.01 | 0.43 | 0.44 |
| DM intake, kg/d | 2.24 | 1.57 | 0.04 | 0.01 | 0.01 | 0.83 |
| Energy balance, Mcal/d | 0.74 | –0.37 | 0.10 | 0.01 | 0.23 | 0.61 |
| Urinary uric acid index | 10.34 | 6.40 | 1.55 | 0.10 | 0.63 | 0.53 |
| Water consumption, L/d | 5.93 | 10.07 | 0.29 | 0.01 | 0.91 | 0.38 |
| Milk yield, kg/d | 1.72 | 1.55 | 0.04 | 0.01 | 0.01 | 0.73 |
| Fat-corrected milk[2], kg/d | 1.94 | 1.65 | 0.03 | 0.01 | 0.01 | 0.75 |
| Milk composition, % | | | | | | |
| Total solids | 12.8 | 11.6 | 0.10 | 0.01 | 0.37 | 0.69 |
| Fat | 4.28 | 3.75 | 0.09 | 0.01 | 0.73 | 0.51 |
| P rotein | 3.77 | 3.25 | 0.08 | 0.01 | 0.23 | 0.26 |
| Casein | 3.23 | 2.82 | 0.06 | 0.01 | 0.01 | 0.90 |
| Milk osmolality, mOsm/kg | 319 | 299 | 4 | 0.01 | 0.04 | 0.17 |

[1] Interaction of treatment (T) × period (P).
[2] Corrected milk yield at 3.5 % fat = L of milk yield × [0.432 + 0.162 × (fat %)].

### 2.6. Statistical analyses

Data were analyzed by the PROC MIXED for repeated measurements of SAS version 9.1.3 (SAS Institute Inc., Cary, NC). The statistical mixed model contained the effects of treatment (TN and HS), period (1 and 2), week (1–4), and interactions of treatment × period, treatment × week, and period × week as fixed effects, as well as the random effects of the animal and the residual error. For rectal temperature and respiration rate measured at 0800, 1200, and 1700 h, a fixed factor of the hour of the day was added to the model. Data of performances (i.e., DMI, water consumption, and milk yield) and physiological indicators (i.e., rectal temperature and respiration rate) were analyzed on weekly average basis. The statistical model of video recording data included an additional fixed effect of daytime (day vs. night). The model considered the possible carryover effects of previous HS periods through the treatment × period interaction. The statistical mixed model of metabolite challenges contained the fixed effects of the treatment (TN and HS) and time relative to metabolite administration; the random effect of the animal; the interaction of treatment × time relative to metabolite administration; and the residual error.

## 3. Results and discussion

### 3.1. Performance variables

Rectal temperatures and respiration rates increased ($P < 0.01$) from 0800 to 1700 h in both goat groups and were greater ($P < 0.001$) in HS than in TN goats (Fig. 1). Our results are similar to that observed in lactating dairy goats from the same breed under similar environmental conditions (Mehaba et al., 2019; Hamzaoui et al., 2021). Goats under HS had the maximum values of rectal temperature and respiration rate during the first 3 days and then decreased ($P < 0.05$), but remained higher ($P < 0.001$) than the TN goats until the end of the experiment. Similar trend over time under HS conditions was observed by Hamzaoui et al. (2013) in dairy goats during late lactation.

The DMI decreased ($P < 0.001$) by 29% because of HS treatment (Table 2). This reduction in DMI is similar to DMI losses in heat-stressed Muraciano-Granadina goats at mid lactation (26–34%; Mehaba et al., 2019; Hamzaoui et al., 2020), but greater than losses observed during late lactation (19%; Hamzaoui et al., 2013). The reduction in DMI because of HS was maximum at d 5 (36%) and partial recovery was observed thereafter (data not shown), but was always lower ($P < 0.001$) than in TN goats. Reduced DMI in heat-stressed animals typically decreases metabolic heat production since heat increment of feeding is an important source of heat production (Sejian et al., 2018). Additionally, the gut fill from water in HS goats might also be related to the reduced DMI. Our HS goats increased their water consumption by 70%, and presumably this was because of the increment in heat dissipation by evaporation (panting and sweating).

In agreement with the depressed DMI, HS goats experienced negative EB (–0.37 Mcal/d), whereas TN goats had a positive EB (0.74 Mcal/d). This negative EB related to HS was also observed in heat-stressed dairy cows (Baumgard and Rhoads, 2013) and goats (Hamzaoui et al., 2020, 2021). In accordance with the DMI and EB data, HS goats lost BW (−45 g/d), whereas TN goats gained BW (+189 g/d on average) during the experimental period. It should be kept in mind that changes in BW of our TN and HS goats included the inevitable variations in digestive tract content.

Heat-stressed goats tended ($P < 0.10$) to have lower UAI compared to TN goats, which might indicate lower microbial synthesis in the rumen of HS goats (Table 2). We admit that using uric acid alone as a proxy of microbial protein synthesis might not be fully accurate since uric acid represents only 10–15% of urinary purine derivatives and some uric acid could be converted to allantoin by uricase (Chen and Ørskov, 2004). However, both urinary uric acid and allantoin respond similarly to experimental treatments and conditions in goats (Lindberg, 1989; Belenguer et al., 2002; Ma et al., 2014), sheep (Puchala and Kulasek, 1992), and cows (Dewhurst et al., 2010). In the current study, the decrease in DMI might explain the reduction in microbial protein synthesis in the rumen of HS goats. Lindberg (1985) noted that urinary purines in goats are highly correlated with DMI. Also, Castro-Costa et al. (2015) reported that heat-stressed goats experience greater rumen temperatures and lower rumen pH, despite having the same DMI values. These changes in rumen conditions because of HS could alter rumen microbial populations. Differences in UAI values were unlikely related to changes in digesta passage rate since Salama et al. (2016) found no significant differences in solid digesta passage rate between TN and HS dairy goats.

Compared to TN, HS goats produced lower ($P < 0.05$) milk yield (10%) and fat-corrected milk (13%) with depressed contents of total solids (9%), fat (12%), protein (14%), and casein (13%) as shown in Table 2. Milk yield losses detected in the current study is within the range (3–13%) reported in heat-stressed dairy goats (reviewed by Salama et al., 2014). In addition, losses in milk components caused by high ambient temperatures have been also observed in dairy cows (Baumgard and Rhoads, 2013) and ewes (Finocchiaro et al., 2005; Mehaba et al., 2021). The negative effects of HS on milk production could be partially explained by decreased DMI in addition to the direct negative effects on the synthetic capacity of the mammary gland. Results in heat-stressed mammary cells showed reduced abundance of genes related to milk fat and protein synthesis (Salama et al., 2019). As already mentioned, UAI tended to decrease by HS, which might decrease amino acids available for milk protein synthesis, since microbial protein represents > 50% of amino acids absorbed in the small intestine (Schwab and Broderick, 2017).

Milk osmolality decreased ($P < 0.01$; Table 2) because of HS, which could be related to increased water consumption (and consequently more diluted milk) in HS compared to TN goats. Heat stress has been found to decrease milk osmolality in Swedish goats (Hartmann et al., 2021) and Friesland sheep (Thompson et al., 1981). However, Mehaba et al. (2021) detected an increase in milk osmolality in HS dairy Lacaune sheep, although HS ewes consumed significantly more water than TN animals. The discrepancy among studies could be related to breed differences in their fluid balance during HS.

**Table 3**
Blood metabolites of Murciano-Granadina dairy goats under thermal neutral (TN, n = 8) and heat stress (HS, n = 8) conditions. Values are least squares means and SE of the mean (SEM).

| Variable | TN | HS | SEM | Effect (*P* <) | | |
|---|---|---|---|---|---|---|
| | | | | Treatment | Period | T × P[1] |
| Insulin, μg/L | 1.17 | 1.42 | 0.286 | 0.54 | 0.87 | 0.46 |
| Glucose, mg/dL | 58.6 | 58.1 | 1.17 | 0.78 | 0.02 | 0.53 |
| Lactate, mmol/L | 0.62 | 0.66 | 0.034 | 0.47 | 0.72 | 0.22 |
| Nonesterified fatty acids, mmol/L | 0.10 | 0.11 | 0.022 | 0.67 | 0.34 | 0.52 |
| β-hydroxybutyrate, mmol/L | 0.49 | 0.55 | 0.045 | 0.39 | 0.39 | 0.42 |
| Blood urea N, mg/dL | 26.6 | 23.0 | 1.2 | 0.06 | 0.01 | 0.87 |
| Creatinine, mg/dL | 0.45 | 0.56 | 0.029 | 0.03 | 0.01 | 0.45 |

[1] Interaction of treatment (T) × period (P).

### 3.2. Blood variables

Despite the marked differences in DMI, there was no difference in blood levels of insulin, glucose, lactate, NEFA and BHB between TN and HS goats (Table 3). Heat-stressed dairy goats (Hamzaoui et al., 2013; 2020; 2021) and ewes (Mehaba et al., 2021) have also been reported to be able to keep similar blood glucose levels to TN animals. The reduced milk yield, and consequently decreased lactose secretion, could spare some glucose in blood since 80–85% of glucose in blood is used by the mammary gland for lactose synthesis in goats (Sano et al., 1985). Also, Hamzaoui et al. (2021) reported that HS reduces milk de novo fatty acids synthesis, and the resultant saved glucose might help HS goats to keep normal blood glucose levels. Additional reasons that may explain how HS goats were able to keep normal blood glucose values are discussed hereafter.

Although HS goats experienced negative EB, blood levels of NEFA did not increase compared to TN goats, which has also been observed in dairy goats (Hamzaoui et al., 2013; Mehaba et al., 2019), dairy ewes (Mehaba et al., 2021), and dairy cows (Baumgard and Rhoads, 2013). The absence of significant difference in blood NEFA values might be related to similar blood insulin levels in TN and HS goats (Table 3). Insulin is a potent lipogenic and antilipolytic hormone (Baumgard and Rhoads, 2013) that prevents body fat mobilization. Insulin is necessary for the activation of the cellular stress response (Li et al., 2006), which might explain why blood insulin levels did not decrease in HS goats even though they ate less and were in negative EB.

Baumgard and Rhoads (2013) reported that HS did not cause significant change in blood NEFA (similar to goats), but increased blood insulin values (such an increase was not detected in goats). Furthermore, Baumgard and Rhoads (2013) indicated that blood lactate levels are consistently elevated in HS animals. Nevertheless, blood lactate did not vary between TN and HS goats in our study. It should be kept in mind that the studies reviewed by Baumgard and Rhoads (2013) were based on the comparison between HS and pair-fed TN animals, whereas our TN goats were fed *ad libitum*.

Blood urea tended ($P < 0.10$) to decrease, whereas creatinine values increased ($P < 0.05$) in HS goats compared to TN animals (Table 3). The decrease in blood urea concentration by HS could be explained by their lower DMI and, consequently, reduced protein intake. On the other hand, the increment in blood creatinine by HS might indicate muscle degradation. Our HS goats were in negative EB (Table 2), but as indicated above, they did not mobilize body fat reserves (no change in blood NEFA as shown in Table 3). However, they mobilized body protein, presumably to use some glucogenic amino acids for gluconeogenesis, resulting in keeping similar blood glucose levels. Heat-stressed dairy cows (Rius, 2018) and ewes (Mehaba et al., 2021) also experience increased blood creatinine levels compared with TN animals.

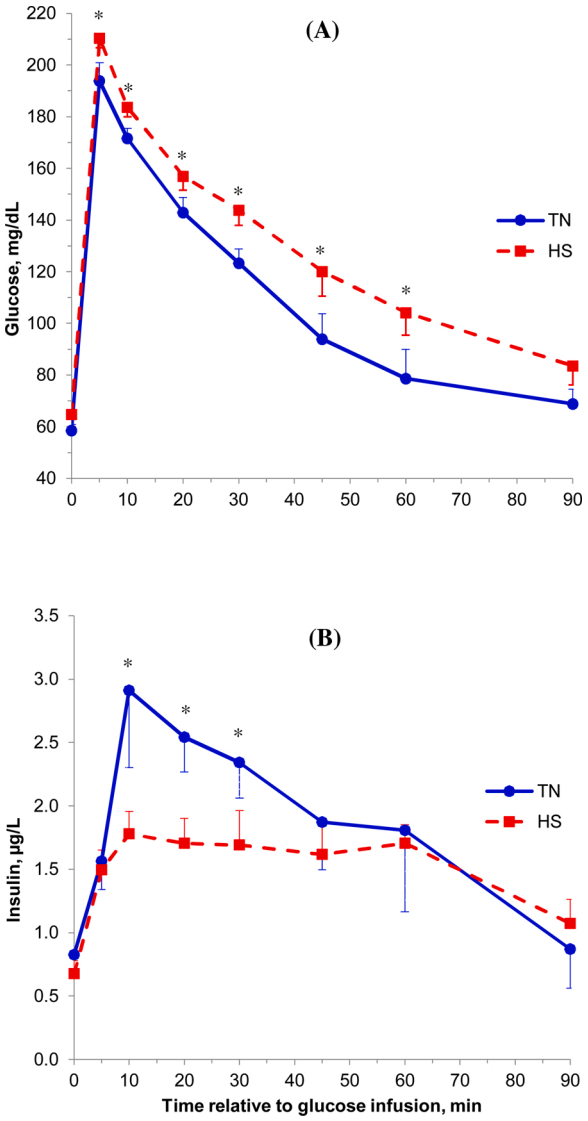


**Fig. 2.** Mean plasma glucose (A) and insulin (B) response to glucose tolerance test of dairy goats under thermoneutral (TN; blue circles) or heat stress (HS; red squares) conditions. * indicates a difference at $P < 0.05$ between TN and HS treatments. SE shown as vertical bars. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

### 3.3. Glucose tolerance test and hormonal challenges

Glucose and insulin responses of TN and HS goats following glucose administration are shown in Fig. 2 and Table 4. Glucose spiked at 5 min in both treatments, but the peak was greater ($P < 0.05$) in HS than in TN goats (Fig. 2a). Blood glucose levels gradually decreased in both treatments with similar clearance rates (1.17%/min on average; Table 4). Glucose AUC was greater ($P < 0.05$) in HS than in TN goats at 30 and 90 min. Mehaba et al. (2021) also found that glucose AUC in response to glucose infusion tends to be greater in HS dairy ewes. Also, Wheelock et al. (2010) reported that lactating HS cows have greater glucose response after glucose infusion than TN cows fed *ad libitum*.

Insulin peaked at 10 min after glucose administration in TN goats, whereas in HS goats a plateau in insulin level was observed from 10 to 60 min, with lower ($P < 0.05$) insulin values at 10, 20 and 30 min (Fig. 2b). This resulted in a tendency of lower ($P < 0.10$) insulin clearance rate and lower ($P < 0.05$) insulin AUC in HS compared to TN goats (Table 4). Thus, the pancreas of HS goats secreted less amounts of insulin in response to glucose infusion compared to TN goats, which could be a

**Table 4**
Metabolite kinetics in response to glucose tolerance test, insulin tolerance test, and epinephrine challenge in dairy goats under thermoneutral (TN; n = 4) or heat stress (HS; n = 4). Values are least squares means and standard error of the mean (SEM).

| Item | TN | HS | SEM | *P*-value |
|---|---|---|---|---|
| Glucose tolerance test | | | | |
| Glucose | | | | |
| Basal, mg/dL | 58.6 | 64.9 | 5.4 | 0.30 |
| Peak, mg/dL | 194 | 211 | 6.6 | 0.05 |
| $AUC_{30\ min}$,[1] mg/L × min | 4450 | 4883 | 171 | 0.05 |
| $AUC_{90\ min}$, mg/L × min | 9586 | 11,367 | 596 | 0.05 |
| $CR_{90}$,[2] %/min | 1.23 | 1.10 | 0.14 | 0.42 |
| Insulin | | | | |
| Basal, μg/L | 0.77 | 0.66 | 0.18 | 0.56 |
| Peak, μg/L | 2.91 | 1.78 | 0.45 | 0.04 |
| $AUC_{30\ min}$, μg /L × min | 69.0 | 47.9 | 7.4 | 0.04 |
| $AUC_{90\ min}$, μg /L × min | 168 | 139 | 29 | 0.36 |
| $CR_{90}$[2], %/min | 1.65 | 0.69 | 0.43 | 0.09 |
| Insulin tolerance test | | | | |
| Glucose | | | | |
| Basal, mg/dL | 59.6 | 59.2 | 2.9 | 0.90 |
| Nadir, mg/dL | 26.5 | 20.0 | 4.9 | 0.24 |
| $AUC_{120\ min}$, mg/dL × min | 5590 | 5223 | 233 | 0.17 |
| ISBGR[3], % | 55.5 | 66.6 | 7.9 | 0.21 |
| Nonesterified fatty acids | | | | |
| Basal, mmol/L | 0.17 | 0.19 | 0.032 | 0.66 |
| Nadir, mmol/L | 0.12 | 0.12 | 0.015 | 0.76 |
| $AUC^2_{120\ min}$, mmol/L × min | 24.3 | 25.7 | 3.9 | 0.74 |
| Epinephrine challenge | | | | |
| Glucose | | | | |
| Basal, mg/dL | 59.3 | 58.3 | 3.1 | 0.78 |
| Peak, mg/dL | 101 | 98.0 | 7.3 | 0.73 |
| $AUC_{120\ min}$, mg/dL × min | 8862 | 9012 | 641 | 0.83 |
| Nonesterified fatty acids | | | | |
| Basal, mmol/L | 0.16 | 0.14 | 0.030 | 0.60 |
| Peak, mmol/L | 0.37 | 0.30 | 0.072 | 0.38 |
| $AUC_{120\ min}$, mmol/L × min | 29.0 | 22.3 | 3.1 | 0.08 |

[1] Area under the curve corrected for the basal levels.
[2] Clearance rate from the peak to 90 min $= \frac{\ln[f_0](\text{glucose at 5 min}) - \ln[f_0](\text{glucose at 90 min})}{90\ \text{min} - 5\ \text{min}} \times 100.$
[3] Insulin stimulated blood glucose response $= \frac{\text{glucose at 0 min} - \text{glucose at 30 min}}{\text{glucose at 0 min}} \times 100.$

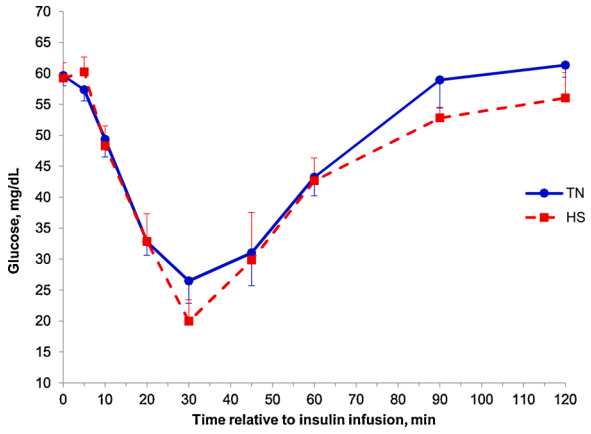


**Fig. 3.** Mean plasma glucose response to insulin tolerance test of dairy goats under thermoneutral (TN; blue circles) or heat stress (HS; red squares) conditions. SE shown as vertical bars. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

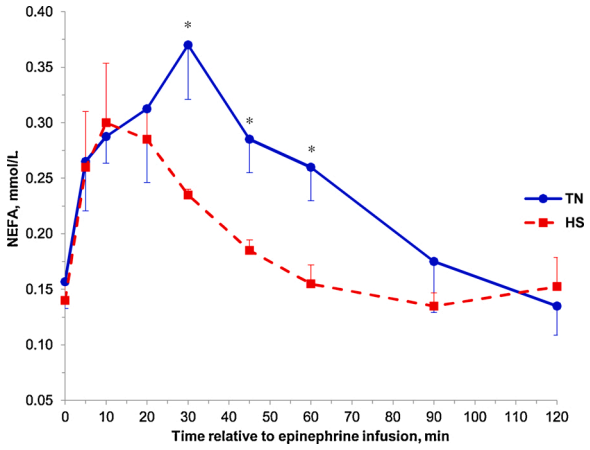


**Fig. 4.** Mean plasma nonesterified fatty acids (NEFA) response to epinephrine challenge of dairy goats under thermoneutral (TN; blue circles) or heat stress (HS; red squares) conditions. * indicates a difference at $P < 0.05$ between TN and HS treatments. SE shown as vertical bars. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

way to keep normal glucose levels in blood under HS despite the reduced DMI (Table 2). Heat-stressed lactating goats (current study; Hamzaoui et al., 2013, 2020) and ewes (Mehaba et al., 2021) experienced decreased DMI, but were able to maintain similar blood glucose levels to TN animals with no change in blood insulin concentration.

Glucose response to insulin administration is shown in Fig. 3 and Table 4. Glucose basal levels averaged 59.4 ± 2.9 mg/dL and were similar in the plasma of TN and HS goats. The level of plasma glucose decreased after insulin (lipogenic signal) administration and reached the lowest ($P < 0.001$) level at 30 min (23.3 ± 4.9 mg/dL). Thereafter, blood glucose concentration increased gradually to basal levels at 90 min and remained unchanged until 120 min. The basal NEFA levels averaged 0.18 ± 0.03 mmol/L before the insulin injection and did not vary between TN and HS goats (Table 4), confirming that no body fat reserves were mobilized in HS goats as mentioned above.

No differences in AUC of glucose and NEFA in response to insulin infusion were observed between TN and HS goats (Table 4). It is well known that insulin causes cells in the liver, skeletal muscles, and fat tissue to absorb glucose from the blood. According to our results, it seems that this happens similarly in TN and HS goats.

Blood glucose peak in response to the epinephrine administration was similar between TN and HS goats (Table 4). Additionally, glucose AUC did not vary, suggesting that the liver of TN and HS goats responded similarly to epinephrine in terms of glycogen breakdown and glucose release. The NEFA response of TN and HS goats following epinephrine administration (lipolysis signal) is shown in Fig. 4. When goats were administered with epinephrine, an increase in blood NEFA was observed in both TN and HS goats. The peak of NEFA in HS and TN goats was observed at 10 and 30 min, respectively. At 30, 45 and 60 min after epinephrine administration, TN goats had greater ($P < 0.05$) blood NEFA levels. This resulted in a tendency ($P = 0.073$) for a lower AUC for NEFA in HS goats (Table 4). A previous study (Collier and Gebremedhin, 2015) showed that although HS is usually accompanied by higher levels of lipolytic hormones as cortisol, no increase in NEFA was detected. Results of the current study clearly indicate that HS induces an insensitivity of lipid body tissues to lipolytic hormones, preventing fat mobilization under high ambient temperature conditions. This finding further explains why NEFA did not vary between TN and HS goats (Table 3). Heat-stressed dairy cows (Baumgard and Rhoads, 2013) and ewes (Mehaba et al., 2021) were also reported to have a reduced NEFA response to epinephrine administration.

**Table 5**
Behavior indices during the day (12 h) and night (12 h) of dairy goats under thermal neutral (TN; n = 8) and heat stress (HS; n = 8) conditions. Values are least squares means and SE of the mean (SEM). Goats in TN were kept at 15-20°C throughout the day, whereas HS goats were at 37°C during the day and at 30°C during the night.

| Items | TN | | HS | | | Effect ($P$ <) | | |
|---|---|---|---|---|---|---|---|---|
| | Day | Night | Day | Night | SEM | Treatment | Daytime | T x D[1] |
| Position change[2], n | 7.6 | 6.6 | 22.6 | 15.1 | 1.8 | 0.01 | 0.02 | 0.06 |
| Standing time, min | 486 | 177 | 278 | 196 | 14 | 0.01 | 0.01 | 0.01 |
| Lying time, min | 234 | 543 | 442 | 524 | 17 | 0.01 | 0.01 | 0.01 |
| Lying time average[3], min | 35.0 | 92.3 | 22.3 | 27.7 | 7.7 | 0.01 | 0.01 | 0.01 |
| Eating bouts, n | 32.1 | 8.8 | 26.0 | 16.4 | 2.3 | 0.82 | 0.01 | 0.01 |
| Eating time, min | 273 | 71 | 105 | 97 | 9 | 0.01 | 0.01 | 0.01 |
| Eating time/meal, min | 8.75 | 8.65 | 4.76 | 6.58 | 3.0 | 0.01 | 0.28 | 0.23 |
| Drinking bouts, n | 10.5 | 2.3 | 21.6 | 8.8 | 2.2 | 0.02 | 0.01 | 0.41 |
| Drinking time, min | 5.8 | 1.3 | 8.4 | 4.4 | 0.94 | 0.05 | 0.02 | 0.88 |
| Drinking time/bout, min | 0.54 | 0.43 | 0.53 | 0.40 | 0.09 | 0.18 | 0.83 | 0.94 |

[1] Interactions of treatment (T) and daytime (D).
[2] Counted as the change from standing to lying and vice versa.
[3] Calculated as total lying time divided by the times of position changing.

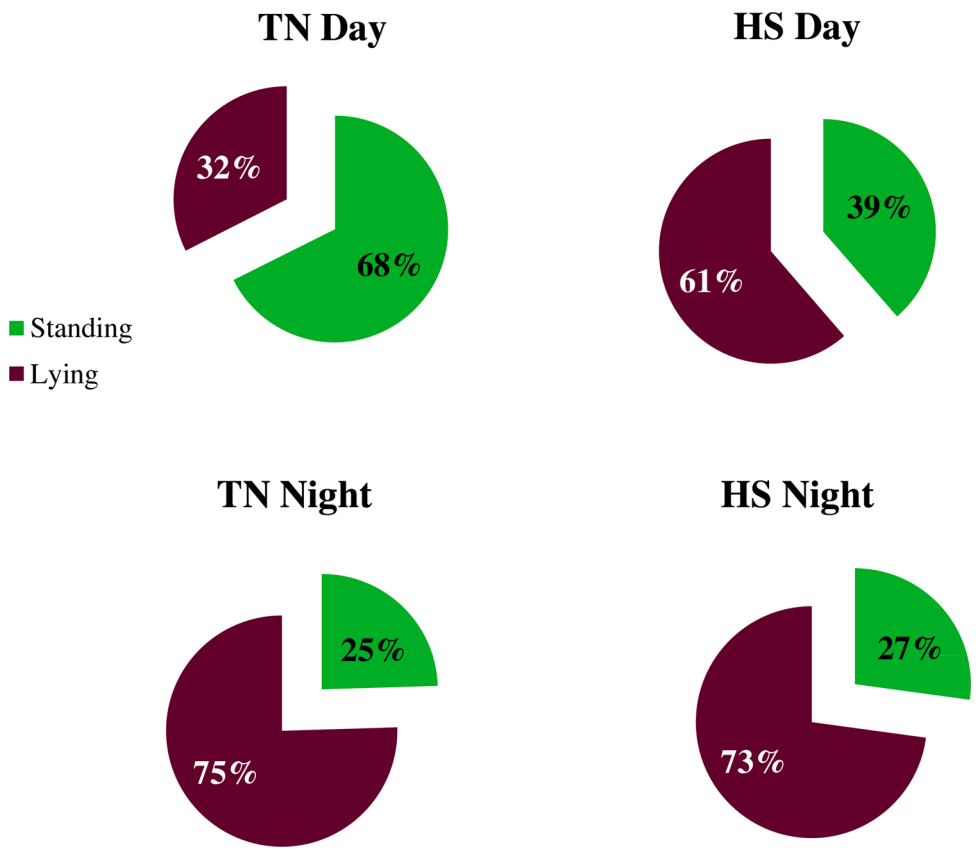


**Fig. 5.** Percentage of time remaining standing or lying down during the day (12 h) and night (12 h) in dairy goats under thermal neutral (TN) or heat stress (HS) conditions.

### 3.4. Behavioral indices

For the behavioral indices in the current study, the day was divided into 2 portions: day during which HS goats were at 37°C with light, and night during which HS goats were at 30°C in dark. The TN goats were at 15–20°C throughout the day with the same light regimen as HS goats. Haley et al. (2000) and Fregonesi and Leaver (2001) reported that the time spent lying down and the duration of individual bouts are sensitive measures of stall comfort and animal welfare. Therefore, information about the duration and frequency of different activities could reflect the comfort of goats under HS conditions.

Observed behavioral indices in TN and HS goats are shown in Table 5 and Figs. 5 and 6. During the day TN goats remained standing (for eating, drinking, or idling) for a longer time than HS goats (68 vs. 39%), while at night, there was no difference between treatments although both spent a shorter time standing (26%) than during the day (Fig. 5). Changing the position (from lying down to standing and vice versa) during day and night was much greater ($P < 0.01$) in HS (18.9 times on average) than TN goats (7.1 times on average). Temple and Manteca (2020) reported that goats in hot environments move more to reorient themselves in different directions to avoid the impact of direct solar radiation and ground radiation. In this current study, both TN and HS goats were in metabolism crates housed within climate chambers (no solar radiation). However, the increment in the frequency of position changing indicates that HS goats were uncomfortable and had extra-movements than TN goats.

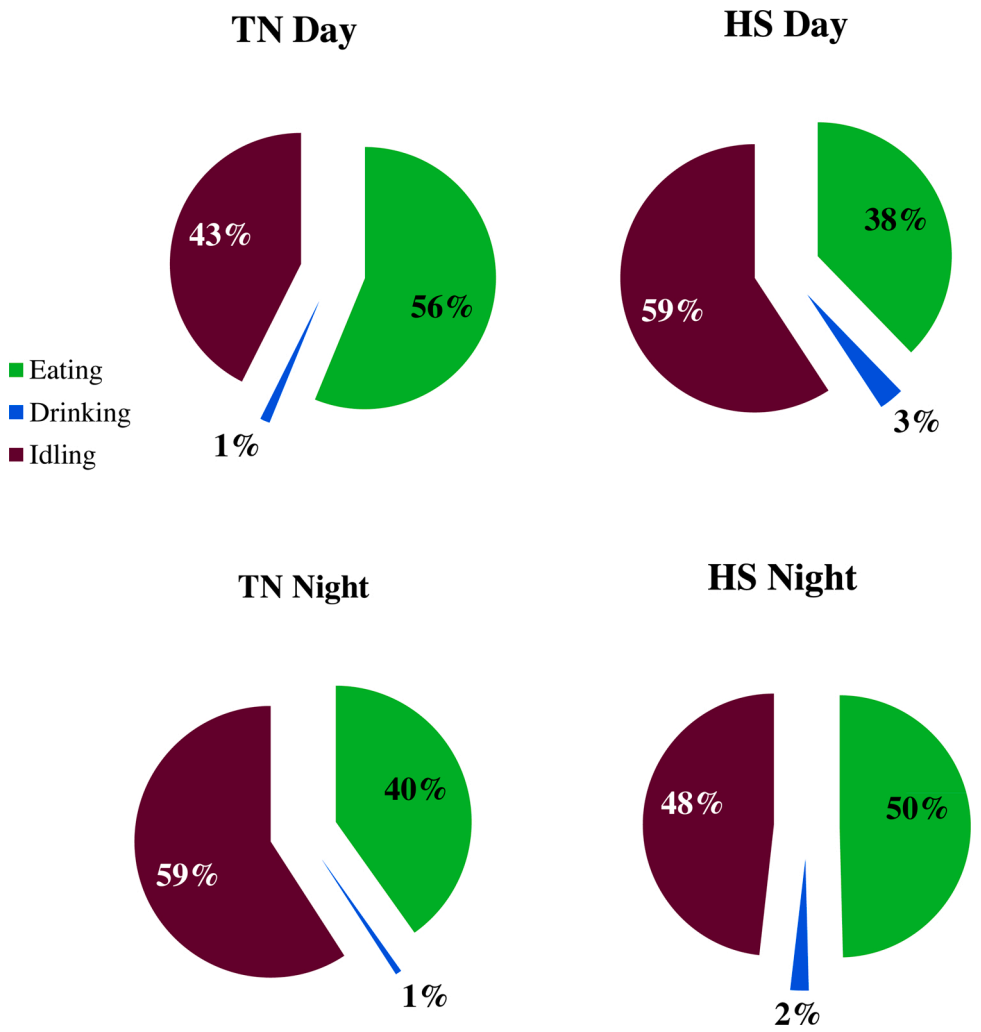


**Fig. 6.** Percentage of time spent while standing devoted to eating, drinking or idling during the day (12 h) and night (12 h) in dairy goats under thermal neutral (TN) or heat stress (HS) conditions.

Total daily eating bouts (41.7) were similar between groups, but greater ($P < 0.001$) during the day (29.1) than during the night (12.6). However, HS goats doubled their number of eating bouts during the night when temperature decreased from 37 to 30°C. Throughout the day, TN and HS had a similar number of eating bouts, but the duration of each meal was shorter ($P < 0.01$) in HS (5.7 min) than in TN (8.7 min) goats (Table 5). This could explain the reduction in DMI observed when goats were subjected to HS. However, the higher water consumption by HS goats could be explained by the greater ($P < 0.01$) number of drinking bouts than in the TN goats (30.4 vs. 12.8) while there was a similar time spent drinking for both groups (0.48 min/bout on average). Cook et al. (2007) showed that total time spent drinking by dairy cows increased from 0.3 to 0.5 h/d when THI increased from 56 to 74.

The percentage of time devoted to different activities (eating, drinking, or idling) while standing are shown in Fig. 6. During the day and in accordance with changes in DMI and water consumption, HS goats spent a greater percentage of time drinking and idling, but a lower portion of time eating than TN goats. During the night, HS goats reduced the portion of time devoted to idling and spent a greater portion of time eating when ambient temperature decreased.

Although our goats were in metabolism cages, time spent lying down by TN goats (13 h/24 h) was similar to the 12–13 h reported for healthy dairy cows housed in a free-stall barn (Cook et al., 2005; Drissler et al., 2005). However, HS goats increased their total lying duration by 3 h (16 h/24 h), but the duration of each lying down was shorter for the HS goats (22 vs. 28 min for day and night, respectively) than for TN goats (35 and 92 min during day and night, respectively) because of the higher frequency of position changing (Table 5). Darcan et al. (2008) and Hartmann et al. (2021) also reported that goats housed in pens and exposed to HS decrease their activity and increase time laying. However, Overton et al. (2002); Zahner et al. (2004), and Cook et al. (2007) reported that the duration of lying behavior decreased with increasing THI in dairy cows. This discrepancy between the goat and cow studies could be because of species differences.

## 4. Conclusions

Exposure of goats to heat stress during mid-lactation reduced milk yield, and fat and protein contents. Although HS goats had lower feed intakes than TN goats, they were able to keep normal blood glucose levels and did not mobilize body fat. Compared to TN goats, HS goats had the same response to insulin, but their adipose tissue was less sensitive to lipolytic signals, which explains the lack of fat mobilization under HS conditions. It seems that the pancreas of HS goats was less sensitive, secreting lower insulin amounts in response to glucose administration. This finding might be a mechanism by which goats keep blood glucose levels stable under high ambient temperatures. Heat stress had no effect on the number of eating bouts, but the observed reduced feed intake for the HS goats was because of a shorter duration of each eating bout. The increase in water consumption under higher ambient

temperatures was because of the higher number of drinking bouts rather than the duration of drinking bouts.

## Declaration of Competing Interest

The authors declare no conflict of interest.

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