Physiological responses and lactational performances of late-lactation dairy goats under heat stress conditions

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ABSTRACT

Eight Murciano-Granadina dairy goats in late lactation were exposed to different ambient conditions, using metabolic cages in a climatic chamber. The experimental design was a crossover (2 periods of 35 d and 4 goats each) and conditions were (1) thermal neutral (TN; 15 to 20°C day-night) and (2) heat stress (HS; 12-h day at 37°C and 12-h night at 30.5°C). Humidity was maintained at 40% and light-dark was constant (12–12 h). The forage:concentrate ratio was adjusted daily for maintaining similar value in TN and HS goats (70:30). Water was freely available at ambient temperature. Rectal temperature and respiratory rate (0800, 1200 and 1700 h) and milk yield were recorded daily, whereas milk composition, nonesterified fatty acids and haptoglobin in blood were analyzed weekly. At d 25, additional blood samples were taken for analysis of metabolites and indicators of the acid-base balance. Digestibility coefficients and N balance were determined (d 31 to 35) and body weight was recorded (d 35). Compared with TN goats, HS goats experienced greater rectal temperature (+0.58°C), respiratory rate (+48 breaths/min), water intake (+77%) and water evaporation (+207%). Intake of HS goats rapidly declined until d 7 (–40%), partially recovered from d 7 to 19, and steadied thereafter (–14%). No changes in digestibility or N balance were detected. Blood nonesterified fatty acids and haptoglobin peaked at d 7 in HS goats but did not vary thereafter. Although milk yield did not vary by treatment, milk of HS goats contained –12.5% protein and –11.5% casein than TN goats. Panting reduced concentration and pressure of CO₂ in the blood of HS goats, but they were able to maintain their blood pH similar to the TN group by lowering HCO₃[–] and increasing Cl[–] concentrations in their blood. In conclusion, HS dairy goats showed dramatic physiological changes during the first week of treatment and partially recovered thereafter. They were able to maintain milk

yield by losing body mass, but milk protein content and protein yield were depressed. Further research is needed to assess the response of dairy goats to HS at earlier stages of lactation.

Key words: heat stress, lactation, digestibility, dairy goat

INTRODUCTION

Heat stress (**HS**) decreases milk production of dairy animals, and half of this reduction in milk yield is due to reduced DMI (Rhoads et al., 2009). The other half of milk yield losses could be explained by the increase in maintenance requirements (NRC, 2007), decreasing secretion of growth hormone (Mittra et al., 1972), lowering blood flow to the udder (Lough et al., 1990), downregulating milk protein genes, and upregulating apoptosis genes in the mammary gland (Collier et al., 2006). Cows under HS had greater levels of insulin with improved insulin sensitivity and lacked the ability of fat mobilization from adipose tissue to face the decreased DMI (Baumgard and Rhoads, 2013). The reduction in dairy farm profit associated with HS when the temperature-humidity index (**THI**) is extremely high is not only a result of decreased milk yield, but also includes impaired milk quality, reproduction problems, increased health care costs, and even animal death.

Despite the large number of studies carried out in dairy cows, little is known about the effects of HS in dairy goats. Goats are considered more tolerant to high THI values compared with dairy cows because of their metabolic size and high water-conservation capacity (Silanikove, 2000). When environmental temperatures increased from 20 to 40°C, respiration rate increased from 30 to over 200 breaths/min in East African goats (Maloiy and Taylor, 1971) and domestic Swedish goats (Olsson et al., 1995), indicating that water evaporation by respiration plays an important role in heat dissipation in goats.

Lactating Saanen goats exposed to moderate or severe HS for 4 d (THI = 81 or 89) lost milk yield by 3 or 13%, respectively (Sano et al., 1985). Brown et al. (1988) reported that the exposure of dairy goats

Received February 4, 2013.

Accepted July 2, 2013.

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to moderate HS conditions for 5 wk (34°C and 25% humidity; THI = 79) depressed milk yield in Alpine but not in Nubian goats, indicating that the response to HS varies according to breed.

The objective of the current study was to measure the physiological, lactational, and nutritional responses to extreme heat stress conditions in Spanish Murciano-Granadina dairy goats in late lactation. Moreover, blood acid-base status and stress indicators were also evaluated. No information is available on the effects of HS on this dairy breed, which is widely spread in the Mediterranean area.

MATERIALS AND METHODS

Animal and Management Conditions

Animal care conditions and management practices agreed with the procedures stated by the Ethical Committee of Animal and Human Experimentation of the Universitat Autònoma de Barcelona (Bellaterra, Spain; CEEAH reference 09/771) and the codes of recommendations for the welfare of livestock of the Ministry of Agriculture, Food and Environment of Spain (Madrid).

Eight open multiparous Murciano-Granadina dairy goats (43.5 ± 2.6 kg of BW) with healthy and symmetrical udders, from the herd of the experimental farm of the Universitat Autònoma de Barcelona were blocked in 2 balanced groups and used at late lactation (194 ± 3 DIM; 1.53 ± 0.04 L/d). The experimental design was a crossover with 2 treatments in 2 periods, lasting 35 d, and 4 goats each. Goats were switched to the opposite treatment in the second period. Climatic conditions were (1) thermal neutral (TN; 15 to 20°C and 45% relative humidity; THI = 59 to 65) and (2) HS (12-h day at 37°C and 40% relative humidity; THI = 85; and 12-h night at 30.5°C and 40% relative humidity; THI = 77). The order of treatments on each goat was recorded and taken into account in the statistical analyses. The THI values were calculated according to NRC (1971) as follows:

$$\text{THI} = (1.8 \times T_{\text{db}} + 32) - [(0.55 - 0.0055 \times \text{RH}) \times (1.8 \times T_{\text{db}} - 26.8)],$$

where T_{db} is the dry bulb temperature (°C) and RH is the relative humidity (%).

Throughout the experiment, (mid-January to mid-April), the TN goats were kept indoors and the temperature was maintained at 15 to 20°C with the help of an electric heater equipped with a thermostat (3.5 kW; General Electric, Barcelona, Spain). The temperature and relative humidity averaged $16.7 \pm 0.3^\circ\text{C}$ and

$45 \pm 5\%$ (THI = 61) for the TN goats. The HS goats were kept in a $4 \times 6 \times 2.3$ -m climatic chamber (Euroshield; ETS Lindgren-Euroshield Oy, Eura, Finland) provided with a temperature and humidity controlling system (Carel Controls Ibérica, S.L., Barcelona, Spain). A continuous $90 \text{ m}^3/\text{h}$ air turnover was maintained throughout the experiment.

Goats had a 4-wk preexperimental period under TN conditions for adaptation to the diet and to metabolic cages. When goats were switched from TN to HS conditions, a transition period of 2 d was allowed (1 d at 25°C and 1 d at 30°C), but no transition was applied for the change from HS to TN. Photoperiod was maintained constant at 12-h light:12-h dark (0900 to 2100 h) and data of environmental temperature and humidity were recorded every 10 min by using 2 data loggers (Opus 10; Luft Mess- und Regeltechnik GmbH, Fellbach, Germany).

Daily ration of the goats consisted of (as-fed) dehydrated fescue hay ad libitum (20% daily refusal), 0.65 kg of alfalfa pellets, and 0.8 kg of concentrate mixture (30% corn, 25.8% barley, 25% soybean meal, 8.5% sunflower meal, 5% FA sodium salts, 2.5% dicalcium phosphate, 2% calcium carbonate, 1% sodium chloride, and 0.2% vitamins A, E, and D₃; as fed). Mineralized salt blocks were freely available in each metabolic cage (composition: 36.74% Na, 0.32% Ca, 1.09% Mg, 5 g of Zn/kg, 1.5 g of Mn/kg, 912 mg of S/kg, 304 mg of Fe/kg, 75 mg of I/kg, 50 mg of Co/kg, and 25 mg of Se/kg; Ovi Bloc; Sal Cupido, Terrassa, Spain). The concentrate mixture was offered in 2 daily portions at 0900 and 1600 h. Changes in the forage intake of the HS goats were taken into account throughout the experiment and the amount of concentrate offered was daily modified to maintain a constant and similar forage:concentrate ratio to that of the TN goats. Clean water was permanently available at ambient temperature, according to treatment.

Goats were milked once daily (0800 h) with a portable milking machine (Westfalia Separator Ibérica SA, Granollers, Spain) set at 42 kPa, 90 pulses/min, and 66% pulsation ratio, provided with recording jars ($2 \text{ L} \pm 5\%$). Milking routine included cluster attachment without udder preparation or teat cleaning, machine milking, machine stripping before cluster removal, and teat dipping in an iodine solution (P3-io shield; Ecolab Hispano-Portuguesa S. L., Barcelona, Spain).

Sample Collection, Analyses, and Measurements

Body Temperature and Respiration Rate. Rectal temperatures and respiration rates were recorded at 0800, 1200, and 1700 h. Rectal temperature was mea-

sured by a digital clinical thermometer (model “mini color”; ICO Technology, Barcelona, Spain; range, 32 to 43.9°C; accuracy, $\pm 0.1^\circ\text{C}$), whereas the number of inhalations and exhalations during 60 s indicated the respiration rate.

Feed Intake and Water Consumption. Feed intake and water consumption (accuracy: ± 20 g) were recorded daily throughout the experiment. Trays with saw dust were put below the drinking troughs and weighted twice daily to take into account water wastes. Feed samples were collected before the beginning of each experimental period and were ground through a 1-mm stainless steel screen, and then analyzed for DM, ADF, NDF, and ash content according to analytical standard methods (AOAC International, 2003). The Dumas method (AOAC International, 2003) with a Leco analyzer (Leco Corp., St. Joseph, MI) was used for N determinations and CP was calculated as percentage of N $\times 6.25$. The chemical composition and nutritive value of ration ingredients are shown in Table 1.

Milk Yield and Milk Composition. Milk yield of individual goats was recorded daily throughout the experiment and milk composition was evaluated weekly. A milk sample of approximately 100 mL was collected and preserved with an antimicrobial tablet (bronopol, Broad Spectrum Microtabs II; D & F Control Systems Inc., San Ramon, CA) at 4°C until analysis. Milk samples were analyzed with a near-infrared spectrometer (Foss NIRSystems 5000; Foss Electric A/S, Hillerød, Denmark) for contents of TS, fat, total protein (N $\times 6.38$), true protein, and CN. Whey protein was calcu-

lated by the difference between true protein and CN, and NPN was calculated by the difference between total protein and true protein.

Blood Measures. Blood samples were taken weekly from the jugular vein into 10-mL plastic lavender Vacutainers with spray-coated K₂-EDTA (BD Diagnostics, Franklin Lakes, NJ) before the morning feeding. Plasma was obtained by centrifugation of whole blood for 15 min at $1,500 \times g$ at 4°C, and stored at -20°C for the NEFA and haptoglobin analyses. The NEFA were determined by the colorimetric enzymatic test ACS-ACOD method using a commercial kit (Wako Chemicals GmbH, Neuss, Germany). Haptoglobin concentration was determined colorimetrically by the hemoglobin-binding method using a commercial haptoglobin assay (Assay phase Range; Tridelta Development Ltd., Maynooth, Ireland) and an Olympus AU400 analyzer (Olympus Europa Holding GmbH, Hamburg, Germany).

At d 25, blood samples (approximately 0.3 mL) were collected using insulin syringes (1 mL; BD Micro-Fine; BD Medical-Diabetes Care, Franklin Lakes, NJ) at 0800 and at 1700 h and immediately analyzed for major ions and metabolites. A single drop of blood was applied to disposable cartridges containing biochemical and silicon chip technology (i-STAT EC8+; Abbott Point of Care Inc., Princeton, NJ). Then, the cartridge was inserted into an i-STAT hand-held analyzer, and the results of glucose, urea, Cl, Na, K, total CO₂ concentration, anion gap, hematocrit, hemoglobin, pH, partial pressure of CO₂, HCO₃⁻, and base excess were obtained.

Table 1. Chemical composition and nutritive value (DM basis) of the ration ingredients used for dairy goats

Item	Fescue hay	Alfalfa pellets	Concentrate
Component			
DM, %	89.99	93.04	90.30
OM, % of DM	89.70	86.50	88.60
CP, % of DM	10.60	12.60	17.90
NDF, % of DM	48.10	44.90	12.60
ADF, % of DM	23.30	27.00	6.19
Nutritive value ¹			
UEm, ² /kg	1.57	—	—
UFL, ³ /kg	0.57	0.68	1.17
NE _L , Mcal/kg	0.97	1.16	1.99
PDIE, ⁴ g/kg	66	83	129
PDIN, ⁵ g/kg	57	95	153
PDIA, ⁶ g/kg	24	47	79
Ca, g/kg	3.5	16.5	18.8
P, g/kg	2.5	2.5	9.1

¹Calculated according to Institut National de la Recherche Agronomique (INRA, 2007).

²Fill units for sheep (1 UEm = 1 kg of reference grass DM).

³Feed units for lactation (1 UFL = 1.7 Mcal of NE_L).

⁴Protein digested in the small intestine supplied by microbial protein from rumen-fermented OM.

⁵Protein digested in the small intestine supplied by microbial protein from RDP.

⁶Protein digested in the small intestine supplied by RUP.

Digestibility Coefficients, and Water and N Balances. Feed orts were daily collected (d 31 to 35), weighed, and composted for analysis. Feces of each goat were daily collected and 10% of fresh feces were dried at 60°C for 48 h. Then, a composted sample for each goat was stored at room temperature until analysis. Urine was collected in containers with 20 mL of H₂SO₄ (96%) and urine volume was daily measured (accuracy: ± 2 mL). Urine samples (5% of total volume) were composted and stored at -25°C for N content analysis. Samples of urine without H₂SO₄ were collected at 0800 and at 1700 h during the last 2 experimental days to measure urine pH. Orts and feces samples were ground through a 1-mm stainless steel screen and then analyzed for DM, CP, CF, ADF, NDF, and ash content, as previously indicated. Water balance was also done during the digestibility period.

Corticosterone in Feces. Samples of fresh feces were collected on the last day of each experimental period and stored at -25°C for corticosterone analysis. Fecal samples were first lyophilized, then extracted with methanol, and finally diluted 1:10 with the assay buffer of the kit. Analyses were performed in the Clinical Biochemistry Service of the Veterinary Faculty of the Universitat Autònoma de Barcelona using the commercially available ¹²⁵I RIA kit (rats and mice corticosterone kit; ICN Pharmaceuticals, Orangeburg, NY), as described by Morrow et al. (2002). Recovery of known amounts of corticosterone added to the processed samples was 59.8%. The relationship between theoretical and true values of corticosterone in goat feces was linear ($y = 0.9919x - 2.863$; $R^2 = 0.997$). The inter- and intra-assay coefficients of variation were 16.8 and 9.5%, respectively.

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 fixed effects of the treatment (HS vs. TN), day, and period; the random effect of the animal; the interactions treatment $\times$ day and treatment $\times$ period; and the residual error. The model took into account the possible carryover effects of previous HS periods through the treatment $\times$ period interaction. Data of performances (i.e., intake, water, and milk yield) and physiological indicators (i.e., rectal temperature and respiratory rate) were analyzed on a daily basis.

For blood parameters measured at 0800 and 1700 h, the model included the effects of treatment, sampling hour, and period, and the interaction treatment $\times$ period and treatment $\times$ hour. Data on digestibility

and nutrient balance were analyzed using PROC GLM of SAS. The model contained the effect of treatment and period, the interaction treatment $\times$ period, and the residual error.

Data were tested for the normality of distribution, and a logarithmic transformation ($\log_{10}$) was applied to haptoglobin concentration in blood. Differences between least squares means were determined with the PDIF test of SAS. Significance was declared at $P < 0.05$ unless otherwise indicated.

RESULTS AND DISCUSSION

Rectal Temperature and Respiration Rate

Rectal temperatures and respiration rates increased from 0800 to 1700 h in both goat groups, but were greater in HS than in TN goats at all time points (Figure 1A and 1B; $P < 0.001$). The increased rectal temperatures and respiration rates in TN goats from 0800 to 1700 h were in accordance with the increment in ambient temperature throughout the day (15 to 20°C). Comparing the HS goats at 0800 h (after being exposed to 30.5°C during the night; THI = 77) with TN goats at 1700 h (THI = 65), we observed similar rectal temperatures ($+0.04^\circ\text{C}$; $P = 0.390$) but greater respiration rates ($+13$ breaths/min; $P < 0.01$), indicating that HS goats were under HS throughout the day, but to a lower extent during the night. Reference respiratory rate for adult goats ranges between 15 and 30 breaths/min according to Pugh and Baird (2012), but it was greater in our TN goats at 1700 h, probably because of breed and physiological state differences. Maximum rectal temperature difference ($+0.70^\circ\text{C}$; $P < 0.001$) between HS and TN goats occurred at 1200 h, whereas the largest difference in respiration rate ($+65$ breaths/min; $P < 0.001$) occurred at 1700 h. Increased respiration rate under HS conditions is a known mechanism for dissipating heat load by evaporation. Rectal temperature and respiration rate values peaked in HS goats during the first week and then gradually decreased, which indicates a partial adaptation to the HS conditions.

Feed Intake

On average, DMI decreased by 21% throughout the 35-d experimental period but showed a marked effect of time elapsed after the start of the HS treatment (Figure 2; $P < 0.001$). Feed intake under HS conditions gradually decreased during wk 1, partially recovered during wk 2 and 3, and remained constant thereafter (Figure 2). Heat stress caused a 27% reduction in DMI from d 1 to 19 (1.47 ± 0.05 vs. 2.00 ± 0.06 kg/d; $P < 0.001$) and 14% from d 20 to 35 (1.75 ± 0.06 vs. 2.03

Figure 1. Rectal temperature (a) and respiratory rate (b) at different hours during the day (0800, 1200, and 1700 h) in dairy goats under thermal neutral (TN; dashed lines; $n = 8$) or heat stress (HS; solid lines; $n = 8$) conditions at late lactation.

± 0.06 kg/d; $P < 0.001$). The partial recovery of DMI from d 19 onwards indicates the adaptation of goats to HS conditions. Previous studies comparing TN and

HS chamber conditions under controlled conditions in dairy cows (Rhoads et al., 2009; Schwartz et al., 2009) did not show such an adaptation.

Figure 2. Milk yield ($\circ$ and $\bullet$), DMI (Δ and $\blacktriangle$), and water consumption ($\square$ and $\blacksquare$) of dairy goats under thermal neutral ($\circ$, Δ , and $\square$ with dashed lines; $n = 8$) or heat stress ($\bullet$, $\blacktriangle$, and $\blacksquare$ with solid lines; $n = 8$) conditions at late lactation. The SEM values of milk yield, DMI, and water consumption are 0.05 L, 0.06 kg, and 1.14 L, respectively.

Feed intake reduction due to HS has been previously reported in dairy goats (Sano et al., 1985), ewes (Abdalla et al., 1993), and cows (Lough et al., 1990; Rhoads et al., 2009). Heat-stressed animals decreased feed intake in an attempt to create less metabolic heat because the heat increment of feeding, especially in ruminants, is an important source of heat production (Kadzere et al., 2002). Moreover, the gut fill by water observed in the current study for HS goats (see later) might also be related to the reduced DMI.

According to the lactational performances of our goats (Table 2) and the requirements estimated according to Institut National de la Recherche Agronomique (INRA, 2007), TN goats showed a greater feed intake (2.03 kg of DM/d or 4.6% of BW) than predicted (1.72 kg of DM/d or 3.9% of BW), which allowed them to cover their daily requirements [1.16 UFL, where UFL = feed units for lactation (1 UFL = 1.7 Mcal of NEL) and 102 g of PDI, where PDI = protein digested in the small intestine] and to have positive energy and protein balances (+0.29 Mcal of NE_L and +80 g of PDI). Moreover, TN goats gained 1.8 kg (+51 g/d) during the experiment, as can be calculated from data shown in Table 2. On the other hand, feed intake of HS goats was lower (1.60 kg of DM/d or 3.7% of BW) than predicted (1.72 kg of DM/d or 3.9% of BW). If we also take into account a 30% increase in maintenance requirements because of HS, as indicated by NRC (2007), the energy

intake would not be enough to cover the daily requirements and resulted in an apparent BW loss of 1.5 kg (−41 g/d). The apparent BW changes in TN and HS goats included the inevitable variations in the digestive tract content, which were unknown in our data.

Water Consumption and Water Balance

Results of water consumption throughout the experiment and water balance measured from d 30 to 35 are shown in Tables 2 and 3, respectively. The HS goats had greater water consumption compared with TN goats (+5.50 L/d; $P < 0.001$). The greatest values of water intake were recorded during wk 1 when DMI was at its lowest value. However, water intake values stabilized earlier than DMI in the HS goats (Figure 2) and remained greater ($P < 0.01$) than in TN goats throughout the experiment. Increased water intake was mainly used by HS goats for boosting heat loss by evaporation from the skin (sweating) and by respiration (panting).

The total water evaporation calculated by subtracting water losses in milk, urine, and feces from water input (water intake + water in food) was 3 times greater (Table 3) in HS goats than TN goats (+2.23 L/d; $P < 0.01$). We were unable to distinguish between evaporation by sweating and panting, but increased sweating rates have been reported in heat-stressed dairy cows (Shwartz et al., 2009) and goats (Baker, 1989).

Table 2. Lactational performance of Murciano-Granadina dairy goats under thermal neutral (TN; n = 8) and heat stress (HS; n = 8) conditions in late lactation [values are LSM and SE of the difference (SED)]

Item	Treatment		SED	Effect (P-value)		
	TN	HS		Treatment	Period	T × P ¹
Initial BW, kg	43.6	44.1	1.3	0.72	0.58	0.83
Final BW, kg	45.4	42.6	2.6	0.034	0.69	0.047
DMI, kg/d	2.03	1.60	0.08	0.001	0.010	0.15
Water consumption, L/d	5.5	11.1	1.20	0.001	0.32	0.47
Milk yield, L/d	1.24	1.21	0.02	0.20	0.001	0.67
3.5% FCM, ² L/d	1.38	1.35	0.07	0.53	0.004	0.23
Milk composition, %						
TS	12.89	12.41	0.29	0.26	0.62	0.73
Fat	4.21	4.22	0.19	0.96	0.90	0.12
Protein	3.84	3.36	0.15	0.030	0.79	0.99
True protein	3.62	3.12	0.14	0.022	0.98	0.96
Casein	3.21	2.84	0.12	0.034	0.18	0.71
Casein, % of protein	84.1	84.9	0.64	0.20	0.001	0.091
Whey protein	0.63	0.53	0.04	0.029	0.001	0.33
NPN	0.22	0.24	0.01	0.16	0.001	0.80
Fat yield, g/d	52	51	2.9	0.68	0.021	0.21
Protein yield, g/d	48	40	1.8	0.001	0.22	0.18
NEFA, ³ mmol/L plasma	0.192	0.137	0.031	0.081	0.67	0.34

¹Interaction of treatment (T) × period (P).²3.5% FCM = L of milk yield × [0.432 + 0.162 × (fat %)].³Average values of blood samples collected weekly from d 0 to d 35.

Milk Yield

Despite the reduced DMI, increased body temperature, and the known negative effect of HS on milk production in dairy cows (West, 2003), milk yield and FCM did not vary between HS and TN goats (Table 2 and Figure 2; $P > 0.05$). Brown et al. (1988) reported that the exposure of dairy goats to constant conditions of 34°C and 25% relative humidity (THI = 79) for 5 wk depressed milk yield in Alpine but not in Nubian goats.

Even when DMI was at its lowest value during the first week, milk yield was not affected in HS goats. During wk 1, HS goats may have been able to partially cover their lactation requirements by body fat mobilization, as indicated by the greater values of NEFA in plasma at d 7 of HS (Figure 3). From d 14 to 28 blood

NEFA levels were similar between groups, but our late-lactation HS goats were probably able to maintain milk yield because they partially recovered DMI, tended to have greater digestibility (see later), and had lower metabolic demands compared with early-lactation goats. Milk yield response of dairy goats to HS in early lactation needs further research.

Our results of NEFA concentrations from d 14 to 28 (but not at d 7) agreed with findings obtained in dairy cows, where HS did not cause an increase in blood NEFA despite the reduced feed intake (Rhoads et al., 2009; Baumgard and Rhoads, 2013). Thus, it seems HS cows are more sensitive to insulin than TN cows, allowing a potent antilipolytic action of insulin that will prevent body fat mobilization. Consequently, lactating HS cows fail to have sufficient glucose for milk synthesis

Table 3. Water input and water losses of dairy goats under thermal neutral (TN; n = 8) and heat stress (HS; n = 8) conditions in late lactation [values are LSM and SE of the difference (SED)]

Item	Treatment		SED	Effect (P-value)		
	TN	HS		Treatment	Period	T × P ¹
Water intake, ² mL	5,504	9,728	1,863	0.035	0.86	0.64
Water in food, mL	143	127	7	0.034	0.010	0.083
Water in milk, ² mL	969	1,004	62	0.55	0.16	0.29
Urine volume, ² mL	2,143	4,757	1,737	0.41	0.99	0.80
Water in feces, mL	1,426	825	144	0.002	0.33	0.18
Evaporation water, ^{2,3} mL	1,074	3,304	1,430	0.007	0.44	0.48

¹Interaction of treatment (T) × period (P).²P-values were extracted after logarithmic transformation because of the nonnormal distribution of data.³Calculated by the difference between water input (water intake + water in food) and water losses in milk, urine, and feces, without taking into account the water produced metabolically.

Figure 3. Plasma NEFA concentrations of dairy goats under thermal neutral (TN; $\circ$; $n = 8$) or heat stress (HS; $\bullet$; $n = 8$) conditions at late lactation. Values are means, with SE indicated by vertical bars. * indicates a difference at $P < 0.001$ between TN and HS treatments.

and, therefore, milk yield was depressed. We did not observe such a decrease in milk yield in our HS goats, probably because the glucose was sufficient for the milk yield level at late lactation (see later).

Milk Composition

Milk TS and fat contents did not vary ($P > 0.05$) between HS and TN goats (Table 2). In short-term studies carried out using climate-controlled HS, milk fat was also not affected in cows (Rhoads et al., 2009; Schwartz et al., 2009) despite the known negative effect of summer on milk fat (Kadzere et al., 2002). This usual decrease in milk fat content of heat-stressed dairy cows during summer might be related to a reduction in forage intake, which could decrease the forage-to-concentrate ratio in the diet. In the current study, concentrate amount was adjusted according to the forage intake in HS goats to maintain a constant forage-to-concentrate ratio throughout the experiment and similar to that of TN goats.

With the exception of milk NPN, the content of protein and protein fractions in milk (true protein, CN, and whey protein) were reduced by HS (Table 2). Nevertheless, the CN-to-protein ratio was not affected. Similarly, dairy cows under controlled conditions of HS decreased their milk protein content (Rhoads et al., 2009; Schwartz et al., 2009). Decreased protein intake and increased sweat secretion that contains protein and urea (Joshi et al., 1968) might have limited the availability of AA for milk protein synthesis. Moreover, the decrease in milk protein content under HS may be due to a lowered microbial protein synthesis in the rumen because of changes in rumen environment (dilution or clearance of soluble substances) by the high water intake. Huber et al. (1994) indicated that under decreased

feed intake conditions, increasing the percentage of RUP and supplementing with lysine increased milk protein content under hot conditions in dairy cows. On the other hand, Bernabucci et al. (2002) suggested that decreased mammary synthesis of milk protein (rather than the reduction in AA intake) is the reason for the low milk protein during the hot season in dairy cows.

Digestibility and Nitrogen Balance

Goats under HS showed numerically greater values of digestibility and tended to have greater ADF digestibility than under TN conditions (+3.1 points; $P < 0.10$), as shown in Table 4. To our knowledge, no data are available comparing digestibility under TN and HS ambient conditions in lactating dairy goats. Our results agree with those of previous research carried out under climatic chamber conditions with male goats (Hirayama et al., 2004), dairy cows (McDowell et al., 1969) and heifers (Bernabucci et al., 1999). The increased digestibility in the HS treatments in the aforementioned studies might be partially due to the reduction of DMI as also observed for HS goats in our results (Table 2). Another reason for the enhanced digestibility under HS conditions could be a depressed passage rate of the solid phase of digesta as reported by Bernabucci et al. (1999). On the other hand, a greater rate of passage of the liquid phase of the digesta may be expected as a consequence of the dramatic increase in water intake. This could have an effect on the availability of rumen soluble fermentable compounds, although this statement needs experimental confirmation. The tendencies observed for an increased digestibility of nutrients (i.e., DM, OM, and ADF; Table 4) in the HS goats might have compensated the reduction in DMI, and could partially explain the lack of effects of HS on milk yield in our results.

Although HS goats had lower N intake (Table 4), as a consequence of the DMI reduction, they experienced lower N losses in feces and urine, which resulted in similar daily N retention in TN goats (13.9 ± 1.0 g/d, on average). Despite the similar N retention, milk protein content was lower in HS than in TN goats, indicating that ingested N would have been directed to other metabolic functions rather than milk protein synthesis in HS goats. It is possible that a portion of N intake was lost in sweat in the form of urea, as previously proposed by Joshi et al. (1968). Moreover, the possibility of a lower supply of essential AA for milk protein synthesis could not be excluded.

Treatment $\times$ period interactions were detected for DM and CP digestibilities, and for fecal excretion and apparent absorption of N (Table 4). These significant interactions could indicate some carryover effect when

Table 4. Digestibility coefficients and nitrogen balance of dairy goats under thermal neutral (TN; $n = 8$) and heat stress (HS; $n = 8$) conditions in late lactation [values are LSM and SE of the difference (SED)]

Item	Treatment			Effect (<i>P</i> -value)		
	TN	HS	SED	Treatment	Period	T $\times$ P ¹
Digestibility						
DM, %	56.6	58.8	2.3	0.12	0.57	0.047
OM, % of DM	58.8	61.2	1.4	0.11	0.41	0.058
CP, % of DM	70.5	72.1	1.5	0.31	0.72	0.021
NDF, % of DM	36.0	38.8	1.9	0.16	0.98	0.76
ADF, % of DM	35.1	38.2	1.7	0.094	0.14	0.60
N balance						
Intake, g/d	48.4	42.1	0.8	0.001	0.012	0.059
Fecal excretion, g/d	14.3	11.8	0.8	0.010	0.35	0.017
Urinary excretion, g/d	20.3	16.4	1.3	0.012	0.96	0.27
Apparent absorption, %	70.5	72.1	1.5	0.31	0.72	0.021
Retention, g/d	13.9	13.9	1.0	0.95	0.16	0.057

¹Interaction of treatment (T) $\times$ period (P).

HS goats went to the TN treatment in the second period. However, this seems unlikely in our case, as these interactions might result from the effect of period on DMI ($P < 0.01$; Table 2), where the DMI reduction in HS goats was more pronounced during period 2 compared with period 1 (data not shown).

Blood Indicators and Urinary pH

Heat stress had no effect on blood glucose, urea, hematocrit, and hemoglobin concentrations (Table 5; $P > 0.05$). A tendency in the treatment $\times$ hour interaction was detected for blood glucose ($P < 0.10$). At 0800 h, HS goats showed lower glucose concentration than TN goats ($P < 0.05$), whereas the concentration at 1700 h increased ($P < 0.001$) and was similar to that of TN goats. It must be stressed that the morning blood samples were taken 1 h before offering the diet, whereas the afternoon sampling was done 1 h after distribution of the second portion of concentrate, which may have contributed to the similar blood glucose levels between groups at 1700 h.

Blood pH is regulated by a complex system of buffers that continuously work to maintain it slightly basic in a range of 7.35 to 7.45 in most mammals (Constable, 1999). Measured blood pH was similar at 0800 in TN and HS goats, but slightly decreased at 1700 h ($P < 0.05$) in the TN goats. Despite the importance of blood pH for understanding the mechanism of respiratory evaporative heat loss, changes observed in our goats were marginally relevant and varied within the normal range in both TN and HS groups.

Values of total CO_2 , partial pressure of CO_2 (pCO_2), HCO_3^- , and base excess were lower in HS compared with TN goats (Table 5; $P < 0.01$). The decreased pCO_2 and HCO_3^- under HS conditions agree with the results reported in dairy cows by Schneider et al. (1988). The

greater respiration rate observed in panting HS goats contributed to a greater loss of CO_2 , lowering the carbonic acid content of the blood. As a consequence, HCO_3^- was transferred from the blood to urine by the kidney to maintain a constant blood pH. Heat stress had no effect on blood Na and K concentrations in accordance with results previously reported in HS dairy cows (Schneider et al., 1988). On the other hand, Cl concentration was greater at both time points in HS than in TN goats (Table 5; $P < 0.05$). Calamari et al. (2007) reported an inverse relationship between blood HCO_3^- and Cl in dairy cows under TN and HS conditions. Due to the greater Cl concentrations of the HS goats, they also have greater anion gap values, compared with TN goats (Table 5; $P < 0.05$). It was expected that the increased HCO_3^- secretion in the urine of HS goats should increase the urine pH, but we observed the opposite, as the urine pH of HS goats tended ($P = 0.11$) to be lower than that of TN goats. We speculated that HS goats were able to increase their renal excretion of H^+ , which resulted in a partial reabsorption of HCO_3^- into the blood, as previously observed by Masero and Siegel (1977). In fact, the decrease in pCO_2 at 1700 h due to HS was more marked (-20%) than the decrease in HCO_3^- (-12%). The secretion of HCO_3^- in urine and its reabsorption suggests a large requirement and turnover of body bicarbonate to maintain blood pH during heat stress.

Haptoglobin Concentration in Blood

Haptoglobin (an acute-phase protein linked to metabolic stress) concentration in blood plasma was greater in HS than TN goats at d 7 ($P < 0.05$), when effects of HS were more marked, but differences between treatment groups disappeared at d 28 (Figure 4; $P > 0.05$). Ametaj et al. (2005) indicated an association between

Table 5. Metabolic and acid-base balance indicators of dairy goats under thermal neutral (TN; n = 8) and heat stress (HS; n = 8) conditions at different daily hours in late lactation (values are least squares means and SEM)

Item	TN		HS		SEM	Effect (<i>P</i> -value)		
	0800 h ¹	1700 h ²	0800 h ³	1700 h ³		Treatment	Hour	T × H ⁴
Glucose, g/L	3.21	3.41	3.04	3.44	0.05	0.197	0.001	0.057
Urea, mg/dL	40.4	30.0	36.5	28.3	2.5	0.331	0.001	0.498
Na, mmol/L	139.5	140.4	142.3	141.0	1.2	0.161	0.881	0.403
K, mmol/L	3.83	4.16	3.70	4.25	0.10	0.793	0.003	0.399
Cl, mmol/L	105.0	108.9	108.3	110.4	0.9	0.031	0.004	0.324
Hematocrit, % PCV ⁵	18.63	17.75	18.25	16.63	0.63	0.372	0.003	0.296
Hemoglobin, ⁶ mmol/L	6.33	6.03	6.23	5.64	0.21	0.363	0.002	0.187
pH	7.42	7.38	7.42	7.42	0.01	0.156	0.142	0.056
Total CO ₂ , ⁶ mmol/L	26.9	24.4	22.3	21.5	0.8	0.002	0.036	0.231
Anion gap, ⁶ mmol/L	12.50	12.00	16.50	14.00	0.73	0.001	0.099	0.428
pCO ₂ , ⁷ mm of Hg	38.9	39.8	33.1	31.5	1.7	0.006	0.769	0.300
HCO ₃ ⁻ , ⁶ mmol/L	25.71	23.41	21.29	20.51	0.83	0.003	0.041	0.283
Base excess ⁶	1.38	-1.75	-3.00	-4.00	0.87	0.005	0.019	0.193
Urine pH	9.09	8.94	8.91	8.85	0.08	0.108	0.215	0.559

¹Before changing from night (30.5°C and 40% humidity; THI = 77) to day (37°C and 40% humidity; THI = 85) conditions.

²During the day (37°C and 40% humidity; THI = 85) conditions.

³Indoor daily variation from 15 (night) to 20°C (day) at 45% relative humidity (THI = 59 to 65).

⁴Treatment (T) × hour (H) interaction.

⁵PCV = packed cell volume.

⁶Calculated values by the i-STAT device software (Abbott Point of Care Inc., Princeton, NJ).

⁷Partial pressure of CO₂.

increased serum haptoglobin concentration and hepatic lipidosis in periparturient dairy cows. Hiss et al. (2009) found that elevated haptoglobin concentrations in milk of dairy cows were associated with high NEFA values in early lactation. This relationship was also observed in our HS goats.

It seems that HS goats at d 7 responded by increased circulating haptoglobin when they were metabolically challenged, as evidenced by reduced feed intake (Figure 2) and greater NEFA concentrations (Figure 3). However, when goats were more adapted to heat stress conditions (i.e., d 28), haptoglobin levels returned to values similar to TN goats.

Corticosterone in Feces

Fecal corticosterone has been used to evaluate stress in cows (Morrow et al., 2002). This approach is based on the fact that glucocorticoids are secreted by the adrenal gland after the activation of the hypothalamic-pituitary-adrenal axis by a stressor. Circulating glucocorticoids are metabolized (conjugated) in the liver and excreted via the urine and feces.

Corticosterone concentrations in feces did not vary between TN and HS goats and averaged 4.28 ± 0.55 ng/g of DM. Similarly, HS did not increase plasma cortisol concentrations in cows (Ei-Nouty et al., 1978) and goats (Olsson and Dahlborn, 1989). Nevertheless, it should be stressed that fecal samples were collected at d 35, and it is possible that differences between TN

and HS goats would have been detected if fecal corticosterone was measured earlier (i.e., at d 7 when HS goats were suffering greater metabolic stress).

CONCLUSIONS

Despite the reduction observed in feed intake of HS goats, they produced similar milk yield to goats under TN conditions in late lactation. However, milk protein content decreased in the HS goats, with no change in milk fat content. Heat-stressed goats had similar N retention to goats under TN conditions, indicating that the ingested N might have been directed to other

Figure 4. Haptoglobin concentrations in plasma of dairy goats under thermal neutral (TN; □; n = 8) or heat stress (HS; ■; n = 8) conditions at late lactation. Values are means, with SE indicated by vertical bars. Means with different letters (a and b) differ (*P* < 0.05).

metabolic functions rather than milk protein synthesis. Further studies are needed to test the HS effects during early lactation and to clarify whether reduced milk protein content is related to a nutrient-limiting factor or reduced mammary protein synthesis, as well as to evaluate the effects on the coagulation properties of the milk.

ACKNOWLEDGMENTS

This work is part of a research project funded by the Spanish Ministry of Economy and Finance (Madrid; Plan Nacional I+D+i; Project AGL2009-09376) and was also supported by a research scholarship to S. Hamzaoui from the International Centre for Advanced Mediterranean Agronomic Studies (Instituto Agronómico Mediterráneo de Zaragoza, Zaragoza, Spain). The authors are also grateful to the team of Servei de Granges i Camps Experimentals (SGCE) of the Universitat Autònoma de Barcelona for the care of the animals.

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