CSIRO PUBLISHING

Animal Production Science
https://doi.org/10.1071/AN19398

Heat stress effects in primiparous and multiparous lactating crossbred cows under a warm environment and their responses to a cooling treatment

J. Castro-Montoya[A] *and E. E. Corea*[B,C]

[A]University of Hohenheim, Institute of Agricultural Sciences in the Tropics (Hans-Ruthenberg-Institute), Animal Nutrition and Rangeland Management in the Tropics and the Subtropics, Fruwirthstrasse 31, 70599, Stuttgart, Germany.
[B]University of El Salvador, Faculty of Agricultural Sciences, Department of Animal Production, Final 25 Avenida Norte, San Salvador, El Salvador.
[C]Corresponding author. Email: elmer.corea@ues.edu.sv

Abstract

Context. In temperate climates, multiparous cows are more susceptible to heat stress (HS) than are primiparous cows. However, these differences in susceptibility may vary in warm environments, where the overall production level is lower, cow size is smaller, and adaptation to HS increases.

Aim. The different effects of HS and its alleviation on primiparous and multiparous cows was tested in crossbred cows (3/4 Holstein × 1/4 Brahman) in a warm environment.

Method. Twelve cows during the rainy season (August–September 2014) and 12 cows during the dry season (March–April 2015; six primiparous, six multiparous) were monitored for rectal temperature and respiration rate, indicators of HS, and milk yield (MY) in a dairy farm in El Salvador. Ambient temperature and relative humidity were recorded hourly to estimate temperature–humidity index throughout the day. During the rainy season, all cows were housed in a pen without cooling treatment. During the dry season, fans and sprinklers were installed in the pen and provided cooling to the herd in two 15-day periods, alternated with two periods without cooling (Control).

Key results. Primiparous cows were more susceptible to HS. Cooling alleviated HS, resulting in an increased feed intake and MY, with a greater impact on primiparous cows. During the cooling treatment, HS parameters increased overnight after the last cooling cycle of the day. This indicated that, despite the cooling treatment, cows still suffered from HS outside the hottest hours of the day. Furthermore, despite a higher temperature–humidity index during the rainy season, rectal temperature and MY remained similar in both the dry and the rainy season for cows without cooling, indicating that animals effectively lower their body temperature by increasing their respiration rate.

Conclusions. The cooling only partially helped alleviate HS in both multiparous and primiparous cows, with the effects being greater on the latter. However, the increments in MY were proportional to the increases in feed intake, indicating that the efficiency of feed utilisation was likely to be not improved.

Implications. More attention should be given to the susceptibility of primiparous cows to HS, as well as to the adjustment of cooling protocols towards alleviating HS in early morning and late afternoon hours.

Keywords: dairy, heat stress, parturition, tropics.

Received 23 July 2019, accepted 10 December 2020, published online 19 January 2021

Introduction

Livestock have several adaptive mechanisms of physical, chemical and ethological responses for the maintenance of homeothermy, but when their capacity to release heat is exceeded, animals will start suffering from what is commonly known as heat stress (HS; Flamenbaum *et al.* 1986). Dairy cows are particularly susceptible to HS due to the large amount of metabolic heat being generated from milk synthesis and feed digestion (Armstrong 1994). Multiparous cows are known to be more susceptible to HS than are primiparous ones, related to the higher level of production of the former and the greater surface area to body mass ratio of the latter (Aguilar *et al.* 2009; Bernabucci *et al.* 2014). However, different from multiparous cows, primiparous cows are still growing during the first lactation, which implies additional metabolic heat production (Lees *et al.* 2019). Under temperate systems, the much greater milk yield in multiparous than primiparous cows produces more metabolic heat than that produced from growth in primiparous cows, leading to an overall greater susceptibility of multiparous cows to HS.

It is also known that HS decreases milk yield (MY) beyond decreases in dry matter intake (DMI), leading to reductions in feed conversion efficiency (FCE). This phenomenon is attributed to changes in the metabolism of carbohydrates, fat and protein, which divert nutrients from growth, reproduction, and production towards the maintenance of homeostasis (Baumgard and Rhoads 2012; Min *et al*. 2017).

In tropical regions, where animals adapted to warm environments have developed mechanisms to cope with higher temperature and humidity, the susceptibility of the cows to HS may differ from that of temperate cattle. For instance, West (2003) highlighted that cows raised at latitudes less than 34°N weighed 6–10% less at birth and had an ~16% lower bodyweight (BW) at maturity than did those in more northern latitudes, even when they were sired by the same bulls. These animals will likely show lower levels of production, with the concomitant less generation of metabolic heat, but a higher basal metabolism (West 2003). Along this line, Ahmed *et al*. (2017) found that cows exposed to HS *in utero* during late gestation have an increased heat tolerance at maturity. High ambient temperature and relative humidity, conditions inherent to tropical regions, may cause a response by the animal towards an adaptation to HS, which is likely being mediated by an increased capacity to dissipate heat to maintain core body temperature (Ahmed *et al*. 2017) and by diminishing milk production (Berman 2011). Moreover, the relative differences in MY and body size of multiparous and primiparous cows are likely to be smaller in warm environments than those in high-yielding cows in temperate systems.

Another important aspect to consider is that animals in tropical regions are exposed to HS throughout the year. However, the conditions conducive of hyperthermia vary between the rainy and the dry season, with higher temperatures in the latter, but higher humidity in the former. Therefore, different susceptibility to HS might appear between both seasons, even for adapted animals (Ribeiro *et al*. 2018). Hence, in the present study, we tested the hypotheses that in warm environments (1) because of their ongoing growth, and the relative smaller differences in MY between primiparous and multiparous, primiparous cows are more susceptible to HS than are multiparous cows; (2) because of impairments in nutrients metabolism under HS, applying a cooling treatment will increase FCE in milking crossbred cattle; (3) because of the higher temperature–humidity index found during the rainy season, cows suffer more from HS during the rainy than during the dry season.

Materials and methods

Experimental site

The study was conducted in the dairy cooperative Astoria in the department of La Paz, El Salvador, a region with precipitation, ambient temperature and relative humidity ranging between 1992 and 2052 mm, 24.2°C and 29.0°C, and 63% and 89% respectively, during the years 2013 and 2014 (Ministry of Environment and Natural Resources 2015). The rainy season spans from May to October, while the dry season lasts from November to April. The experimental procedures applied to the cows were approved by the Research Council of the University of El Salvador and the Board of Directors of the Faculty of Agricultural Sciences in the agreement no. 64/2011–2013 (V.4), which is the authority in this matter. The approval was further ratified by the board of directors of the Astoria cooperative.

Experimental animal management and measurements

The cows selected for the study belonged to a herd of 60 milking cows with predominantly Holstein genetics (3/4 Holstein, 1/4 Brahman). The herd was housed in a concrete-floored 30 × 20 m pen, with a shaded area of 20 × 10 m (main pen; Fig. 1). The cows were fed a total mixed ration consisting of 14.6 kg DM sorghum silage and 6.3 kg DM of concentrate (Table 1). The diet was designed using the CPM dairy software V3.08. The N concentrations were determined in dried total mixed ration samples by Kjeldahl procedure, using digestion DK and distillation UDK 129 units (VELP Scientifica, Usmate, Italy). Crude protein was estimated by multiplying N concentrations by 6.25 (Method 990.13, AOAC International 2005). Concentration of NDF (inclusive of residual ash) was determined using heat-stable α-amylase (Van Soest *et al*. 1991), by using an Ankom 200 fibre analyser (ANKOM Technology, Macedon, New York, USA). The feed was offered at 0700 hours, 1000 hours, 1400 hours and 1700 hours, with *ad libitum* access to water. Milking was undertaken twice a day at 0300 hours and 1500 hours, by using an automated milking system (Fullwood, Shropshire, UK). Measurements of ambient temperature (AT) and relative humidity (RH) as well as MY, and indicators of HS, occurred at two different

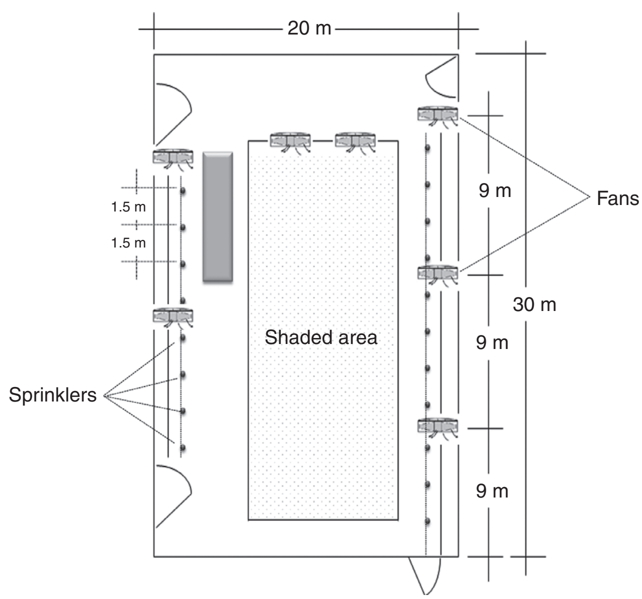

Fig. 1. Technical design and distribution of the cooling system (i.e. fans and sprinklers) in the main pen to provide heat stress alleviation to the herd of lactating dairy cows during the dry-season trial.

Table 1. Ingredient composition of the total mixed ration used in the study and concentrations of crude protein, neutral detergent fibre and metabolisable energy

Item	Concentration
Ingredients (g/kg dry matter, DM)	
Sorghum silage	700
Sugarcane molasses	54.0
Cornmeal	52.5
Wheat bran	45.0
Dried distiller's grains	42.0
Palm kernel cake	30.0
Rice bran	24.0
Coffee hulls	21.0
Soybean meal	18.0
Calcium carbonate	6.0
Sodium chloride	2.4
Urea	2.1
Dicalcium phosphate	1.5
Minerals mix	1.5
Chemical composition	
Crude protein (g/kg DM)	111
Neutral detergent fibre (g/kg DM)	502
Metabolisable energy (MJ/kg)[A]	10.1

[A]According to software CPM dairy V3.08.

moments, namely, during the rainy season (August and September 2014) and during the dry season (March and April 2015). The original experimental design of the study considered using the cooling system to alleviate HS during both seasons; however, due to a delayed arrival of the equipment, this was possible only during the dry season.

In the rainy season, 12 milking cows (6 primiparous and 6 multiparous) with normal health histories were selected for monitoring of MY, respiration rate (breaths/min) and rectal temperature (°C) in four consecutive days in August, and 2 weeks later in 4 days in September. The cows had an average liveweight of 514 ± 35 kg, daily MY of 16.3 ± 1.4 kg, and 92 ± 29 days in milk. AT and RH were recorded hourly over the 24 h by using a portable humidity and temperature meter (VWR Traceable, PA USA). Rectal temperature and respiration rate were measured at 0900 hours, 1100 hours, 1300 hours, 1500 hours and 1700 hours, by means of a digital thermometer and by counting abdominal movements for 30 s respectively.

Temperature–humidity index (THI) was estimated according to Hahn (1999), as follows:

$$\mathrm{THI} = 0.81 \times \mathrm{AT} + \mathrm{RH}/100 + (\mathrm{AT} - 14.4) + 46.4, \quad (1)$$

where AT = ambient temperature (°C), and RH = relative humidity (%).

In the dry season, four experimental periods of 15 days each (11 days of adaptation and 4 days of measurements) were used to monitor the same parameters as in the rainy season, but two experimental periods included the activation of a cooling system. Therefore, within the dry season, in Periods 1 and 3, no cooling (i.e. Control) was applied to the herd, whereas in Periods 2 and 4, the cooling system (i.e. Cooling) was activated. For this, 220 V, 91.5 cm diameter fans and water sprinklers were installed over the two linear feed troughs in the main pen (Fig. 1). The fans were placed every 9 m, with an inclination of 30° towards the ground. Two additional fans were placed in a shaded rest area. Water sprinklers were placed at a height of 2 m and 1.5 m apart (Fig. 1). Following normal practices for dairy farming in El Salvador, the cooling protocol consisted of four 1-h cooling cycles applied during the hottest part of the day, i.e. at 1000 hours, 1200 hours, 1400 hours and 0100 hours each day during the cooling periods. Within a cycle, the sprinklers were set to open every 10 min for 2 min with a release volume of 2 L/min, while the fans were activated throughout the 60 min with an air velocity (9 m far from the fan) of 2 m/s. Twelve cows that were different from those used in the rainy season (6 primiparous and 6 multiparous) were selected from the main herd for individual measurements. These animals had an average liveweight of 520 ± 35 kg, a daily MY of 17.0 ± 1.2 kg, and 84 ± 26 days in milk. DMI was estimated only during the dry season. However, because of the impossibility of obtaining individual measurements of intake, DMI was estimated on the main-herd basis by weighing feed offered and refusals over the measurement period. An averaged DMI was assumed for each animal. MY was registered daily for each cow during the 4 days of data collection.

Statistical analyses

The effects of cooling treatment on respiration rate and rectal temperature for each measuring time, and for daily MY were evaluated with the MIXED procedure of SAS, by using dry-season measurements with the underlying model

$$Y_{ijk} = \mu + T_i + P_j + (T \times P)_{ij} + R_k + \varepsilon_{ijk}, \quad (2)$$

where Y_{ijk} = dependent variable, μ = overall mean, T_i = fixed effect of the *i*th treatment (e.g. control, cooling), P_j = fixed effect of the *j*th parity (i.e. primiparous, multiparous), $(T \times P)_{ij}$ = fixed interaction effects between treatment and parity, R_k = random effect of the *k*th cow, and ε_{ijk} = error term.

The effects of thermal treatment on DMI were evaluated on the group basis, using the daily average as replicate in a mixed model as follows:

$$Y_{ij} = \mu + T_i + R_j + \varepsilon_{ij}, \quad (3)$$

where Y_{ijk} = dependent variable, μ = overall mean, T_i = fixed effect of the *i*th treatment (e.g. Control, Cooling), R_j = random effect of the *j*th day, and ε_{ij} = error term.

Additionally, with the objective to evaluate the differential responses of primiparous or multiparous cows to HS during either dry or rainy season, regression slopes were estimated for the parity–season combination by regressing respiration rate or rectal temperature on each of AT, RH and THI, including the three-way interaction with parity and season, by using a random model with cow as repeated-measurement in the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA).

For all statistical analyses, differences were declared at $P < 0.05$, and tendencies were assumed if $0.05 < P < 0.10$. Mean differences were estimated by performing a Tukey's test.

Results

AT, RT and THI

Average AT during the dry season (33.5 ± 2.18, min = 29.0, max = 38.0°C) was higher than that during the rainy season (32.5 ± 1.65, min = 29.2, max = 36.1°C; Fig. 2*a*). Expectedly, RH was higher during the rainy season (66.8 ± 6.36, min = 62.1, max = 87.0%) than during the dry season (48.8 ± 9.84, min = 42.0, max = 79.0%; Fig. 2*a*), which caused a higher THI during the rainy season (84.8 ± 1.87, min = 76.6, max = 87.5) than in the dry season (82.8 ± 2.43, min = 75.8, max = 84.6), peaking at ~1300 hours (Fig. 2*b*).

Respiration rate and rectal temperature

Respiration rate during the control period was higher in primiparous than in multiparous cows ($P < 0.01$; Fig. 3). The cooling treatment successfully decreased respiration rate in both primiparous and multiparous cows ($P < 0.01$). However, respiration rate at 0900 hours, before the first cooling cycle of the day, remained unaffected ($P = 0.90$). An interaction effect showed that cooling caused more pronounced decreases in respiration rate for primiparous than for multiparous cows ($P < 0.05$; Fig. 3), leading to a similar respiration rate between primiparous and multiparous cows when cooling was applied to the herd ($P > 0.05$). Rectal temperature (Fig. 4) was higher for primiparous than multiparous cows and for the control than the cooling treatment ($P < 0.05$). No parity × cooling interactions were observed, but different from the respiration rate, primiparous cows in the control periods at 0900 hours had a higher rectal temperature than in all other parity × treatment combinations.

DMI and MY

Dry matter intake during the dry season on the main-herd basis increased when the animals were under the cooling treatment ($P < 0.01$; Table 2). Similarly, MY increased when cooling was applied ($P < 0.01$), with larger increases for primiparous (9.7%) than for multiparous (6.5%) cows (Table 2). No

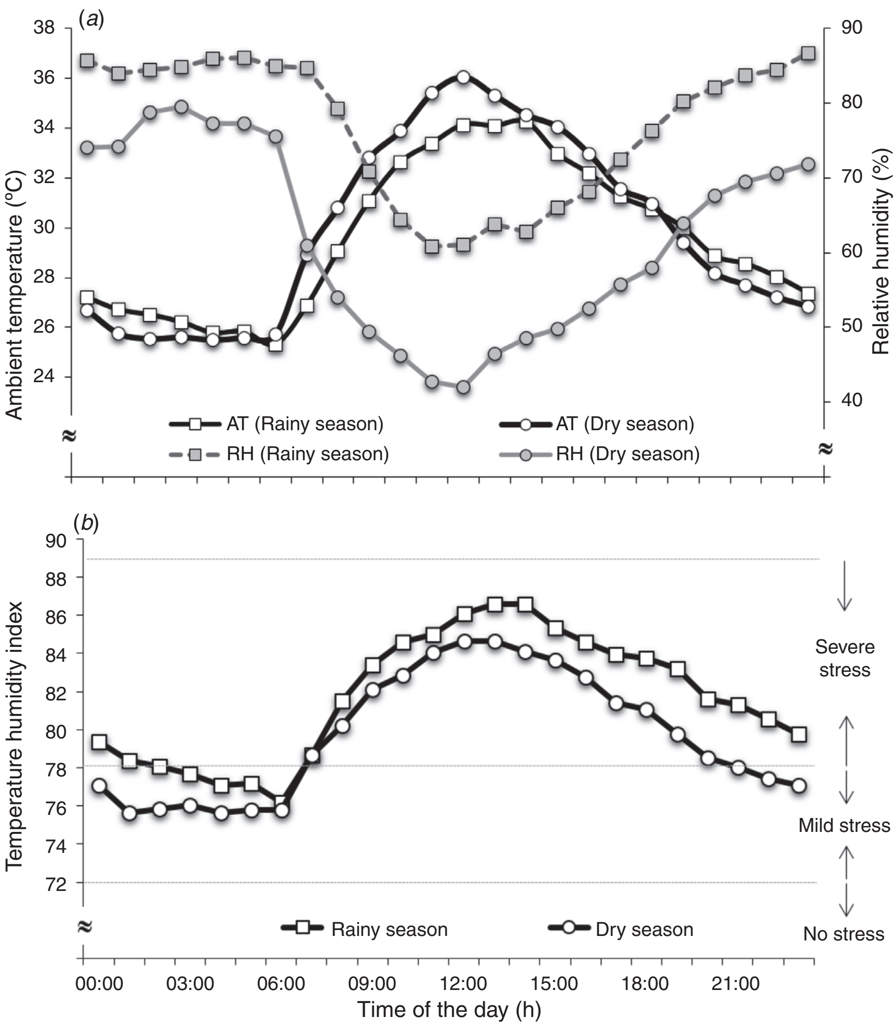

Fig. 2. (*a*) Ambient temperature (AT) and relative humidity (RH) and (*b*) temperature–humidity index throughout the day during the measurement periods for the dry and the rainy season. Dotted lines in *b* indicate thresholds of heat stress (Moran 2005).

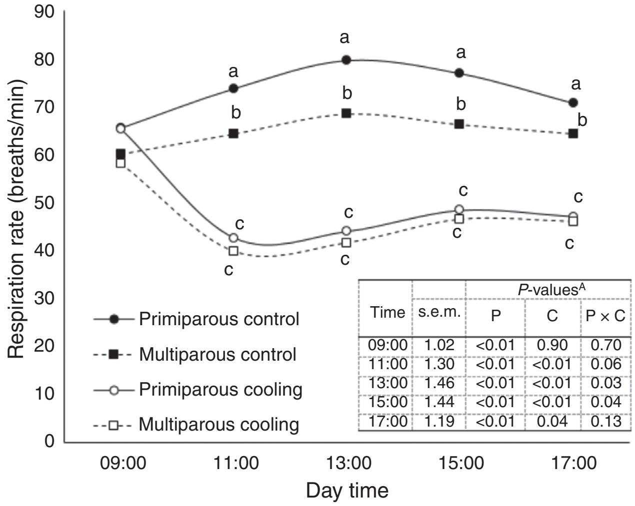

Fig. 3. Respiration rate of heifers and cows with or without cooling treatment for heat stress alleviation during the dry season in a warm environment. P, effect of parity; C, effect of cooling; P × C, interaction effects between parity and cooling.

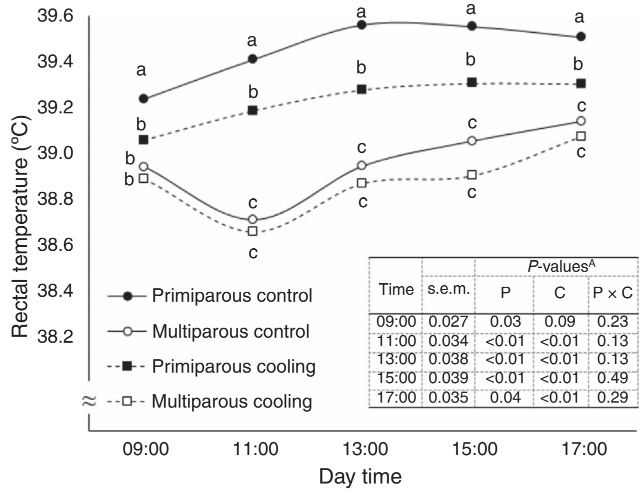

Fig. 4. Rectal temperature of heifers and cows with or without cooling treatment for heat stress alleviation during the dry season in a warm environment. P, effect of parity; C, effect of cooling; P × C, interaction effects between parity and cooling.

Table 2. Dry matter intake and milk yield of primiparous and multiparous cows with or without cooling treatment for heat stress alleviation during the dry season in a warm environment

Dry matter intake was measured on the herd basis, without separation of primiparous and multiparous cows. P, effect of parity; C, effect of cooling; P × C, interaction effects between parity and cooling. Within a row, values followed by different letters are significantly different (at $P < 0.05$)

Variable	Control		Cooling		s.e.m.	*P*-value		
	Primiparous	Multiparous	Primiparous	Multiparous		P	C	P × C
Dry matter intake (kg/day)	17.7		19.4		2.23	—	<0.01	—
Milk yield (kg/day)	14.4d	16.9b	15.8c	18.0a	1.86	<0.01	<0.01	<0.01

differences were observed for MY between cows in the rainy and those in the dry season with no cooling treatment (16.9 and 14.4 kg for multiparous and primiparous versus 17.6 and 15.1 kg for multiparous and primiparous cows, during the dry and the rainy seasons respectively; $P > 0.05$).

Effects of environmental conditions during the dry or rainy season on HS parameters for cows without cooling treatment

Across all data, rectal temperature was positively related with RH, AT and THI ($P < 0.05$). Greater increases in rectal temperature were observed on primiparous than on multiparous cows (Table 3). Furthermore, RH had a greater effect on rectal temperature during the dry season ($P < 0.01$), whereas AT had a greater effect on rectal temperature during the rainy season ($P < 0.01$). Rectal temperature of primiparous cows over both seasons was not affected by THI ($P > 0.05$), but for multiparous cows, THI caused a higher rectal temperature during the dry season ($P < 0.05$; Table 3).

Respiration rate was negatively related with RH and positively related with AT and THI ($P < 0.05$; Table 3). The effects of RH on respiration rate were greater during the rainy season than during the dry season for both multiparous and primiparous cows ($P < 0.01$). AT and THI also showed a greater effect on respiration rate of primiparous cows during the rainy season ($P < 0.05$), but not for multiparous cows, where AT and THI had similar effects in both seasons ($P > 0.05$; Table 3).

Discussion

Even though the THI has to be interpreted in the context of factors such as genetic tolerance for heat, animal size, and MY-related metabolic heat production, it is safe to assume that the animals in this experiment were under moderate to severe HS. Armstrong (1994) mentioned that at THI beyond 79 HS leads to reductions in DMI, accompanied by an increased body temperature severely affecting productive and reproductive performance, whereas Moran (2005) stated that tropical cattle suffer from severe stress at THI of >78.

Differences between primiparous and multiparous cows

During the dry season, primiparous and multiparous cows were subjected to a cooling treatment aiming at evaluating the effects of HS alleviation on respiration rate, rectal temperature and MY. Primiparous cows are usually expected to suffer less from HS due to their lower production than that of multiparous ones; the former generate less metabolic heat, while also having a larger

Table 3. Regression coefficients of the regressions of rectal temperature and respiration rate on relative humidity, ambient temperature and temperature–humidity index of cows and heifers during the dry or the rainy season with no cooling treatment (n = 960)

Number of observations was derived from hourly measurements per cow and period. Slope values followed by different letters are significantly different (at P = 0.05). X is the slope of the overall model for the independent variable

Variable	Intercept	Slope (s.e.)				
		X	Multiparous dry season	Multiparous rainy season	Primiparous dry season	Primiparous rainy season
			Relative humidity (X)			
Rectal temperature	39.1 (0.11)	0.0034 (0.0025)	0c	–0.0026d (0.0011)	0.0050a (0.0010)	0.0028b (0.0011)
Respiration rate	67.8 (3.11)	–0.069 (0.0702)	0d	0.076c (0.033)	0.187b (0.030)	0.236a (0.033)
			Ambient temperature (X)			
Rectal temperature	4.57 (7.92)	1.75 (0.23)	0c	0.211b (0.041)	0.255b (0.039)	0.542a (0.041)
Respiration rate	37.7 (0.28)	0.042 (0.0084)	0c	–0.0007c (0.0014)	0.0067b (0.0013)	0.0106a (0.0014)
			Temperature–humidity index (X)			
Rectal temperature	35.3 (0.56)	0.047 (0.0068)	0b	–0.0028c (0.0005)	0.0027a (0.0005)	0.002a (0.0005)
Respiration rate	–73.7 (15.67)	1.67 (0.189)	0c	0.0078c (0.0163)	0.1046b (0.0158)	0.1349a (0.0163)

surface area compared with internal body mass (West 2003; Aguilar *et al*. 2010). This has been confirmed in studies evaluating genetic variation among Holstein cows by using thousands of daily milk records (Aguilar *et al*. 2009; Bernabucci *et al*. 2014), where a greater HS-driven decrease in MY in cows of third parity than in primiparous cows has been found. This contrasts with our findings, where primiparous cows were more affected by HS than were multiparous ones. Two factors could be the main determinants of this discrepancy. First, heifers in this trial weighed, on average, 503 ± 49.3 kg, while multiparous cows weighed 537 ± 49.8 kg, a difference of 0.69 standard deviations, which is much smaller than that of high-yielding milking cows in herds of, for example, Canada (568 ± 47.0 kg and 674 ± 54.7 kg, for primiparous and multiparous cows respectively; a difference of 2.04 standard deviations; Neave *et al*. 2017), or Brazil (571 ± 72.7 and 677 ± 74.2 kg, for primiparous and multiparous cows respectively; a difference of 1.44 standard deviations; Poncheki *et al*. 2015), indicating that the advantage of primiparous cows to dissipate more heat due to their greater surface area to body mass ratio would have been minimised in the current study. Second, the differences in MY between primiparous and multiparous cows in the present study were also small (2.35 kg) compared with differences in high-yielding cows (~up to 10 kg/day; e.g. Wathes *et al*. 2007; Neave *et al*. 2017). These smaller relative differences in MY and BW most likely play a role on the amount of heat generated by cows of different parities.

Inevitably, a proportion of the metabolisable energy (ME) needed for growth or milk production is lost as heat. The synthesis of 1 kg of milk requires ~5.5 MJ ME with an efficiency (k_l) of ~0.6, whereas the production of 1 kg of gain (muscle and fat) requires ~44 MJ ME with an efficiency (k_g) of ~0.5 (Society of the Physiology of Nutrition 2011). On the basis of the figures presented in the previous paragraph, in a scenario where a high-yielding milking cow produces a delta of 10 kg of milk, a multiparous cow needs 55 MJ of ME, of which 22 MJ is lost as heat. In contrast, a primiparous cow that gains 100 kg BW within 1 year would be gaining roughly 0.27 kg daily, needing 11.9 MJ ME, of which 5.9 MJ would be lost as heat. The difference in heat losses between additional milk from multiparous and additional BW gain from primiparous cows is 16.1 MJ/day, which represents the additional heat generated by high-yielding multiparous cows compared with primiparous ones. By following the same rationale and calculations and considering a difference in MY of 2.5 kg higher for multiparous and a BW gain of 0.1 kg daily for primiparous (~35 kg BW gain in 1 year), the difference in heat generation from both processes would be only 3.3 MJ/day more for multiparous cows. Obviously, there are factors that would affect the accuracy of these calculations (e.g. precise ME requirements, precise efficiency, level of production, DMI level, digestion, rate of weight gain, body size, activity), but they certainly reflect the minimised difference in heat generation from production (milk or growth) between primiparous and multiparous cows under the conditions of the present study.

The above might indicate that when comparing primiparous and multiparous cows of a similar size and level of production, the former would be disadvantaged due to their additional need of energy for growth and, possibly, because of their lessened adaptation to the stress of lactation, which carries metabolic changes to which multiparous cows are likely to have already adapted. Moreover, even though Bernabucci *et al*. (2014) found greater decreases in MY in multiparous cows under HS than in primiparous ones, protein concentration in milk showed a greater HS-driven reduction in primiparous than in multiparous cows, which was likely to be related to the additional need of protein by the former to sustain their growth (Wathes *et al*. 2007). Along this line, Cowley *et al*. (2015) stated that under HS, amino acids might be used for gluconeogenesis rather than for milk synthesis (or muscle accretion). Both the statements of Wathes *et al*. (2007) and Cowley *et al*. (2015) may further confirm the greater susceptibility of primiparous cows to HS under the conditions of small differences in production and body size, than that of with multiparous.

Effects of cooling treatment

The cooling treatment during the dry season effectively alleviated HS in the dairy cows in the current study, with greater effects on primiparous cows, likely due to their higher susceptibility to HS. Rectal temperature decreased by 0.4°C and 0.3°C in primiparous and multiparous cows respectively, and respiration rate decreased by 23.2 and 17.6 breathes/min in primiparous and multiparous cows respectively. The alleviation of HS is reflected in an increased MY and DMI. DMI decreases with HS as a response by the animal to reduce heat production; the lower feed intake leads to a decreased gluconeogenesis, resulting in less glucose available for lactose synthesis in the mammary gland, which expectedly leads to a reduction in MY (Lohrenz *et al*. 2010). However, it has been confirmed that under HS, the decreases in MY occur beyond the reduced nutrient intake, mainly due to an impaired carbohydrate metabolism, where available glucose is preferentially used in processes other than milk synthesis (Streffer 1988; Wheelock *et al*. 2010; Baumgard *et al*. 2011). This explained that when cows without HS were fed at the same intake level as were cows under HS, the former still produced more milk than did the latter (Wheelock *et al*. 2010).

A higher FCE by cows under a cooling treatment was not observed in the current study, as both intake and MY increased in a similar proportion when the cooling system was applied (FCE of 0.88 and 0.87 kg milk/kg DMI during the control and the cooling periods respectively). However, the reported FCE must be interpreted carefully, because only measurements of DMI at the herd level were available, an issue that the reader must keep in mind for the following discussion. Still, the divergence between the current findings on FCE and available literature on high-yielding cows suffering HS are still remarkable and deserve further discussion. Plausible explanations for such contrast are listed here. (1) It is possible that the effects on nutrients metabolism appear only from a certain level of HS that the cows in the current study did not achieve, which could be due to their much lower amount of metabolic heat generated from production than for high-yielding cows, and their adaptation to the warm climate. (2) The cows used in the current study were crossbreds containing a proportion of *Bos indicus* cattle, whose abilities to regulate rectal temperature are well known (Finch 1986); additionally, Colditz and Kellaway (1972) found that when comparing Friesians, Brahmans and their F_1 crosses exposed to a warm environment, rectal temperature decreased with increasing proportion of Brahman, but the DMI decline was much smaller for the F_1 crosses (1.4%) than for the pure breeds (17% and 12% for Friesians and Brahmans respectively). The previous statement may support the idea that the DMI decline in the crossbred animals under HS in this study might not be as steep as it could be expected from the knowledge based on pure breed cows. (3) Another possibility resides in the other end of the spectrum, where the cooling treatment could have alleviated the effects of HS regarding voluntary intake, but not at the metabolic level. This is a possibility considering that for measurements at 0900 hours, there was no effect of cooling for respiration rate and only a tendency for rectal temperature, indicating that after the last cooling cycle (1700 hours) the cows started again suffering from HS for the next 17 h at the same level as during the control periods. The discrepancies observed between the findings of the current study and the well established effects reported by Baumgard *et al*. (2011) and Wheelock *et al*. (2010) indicated a need for further research on the effects of HS on FCE in cattle adapted to warm regions.

It is important to mention that due to the experimental design and the impossibility to separate primiparous from multiparous cows at the time of feeding, in the present study, it was assumed that the effects of HS and its alleviation on DMI were similar between both cohorts. The reduction of DMI itself is a mechanism by which cattle cope with HS (Berman 2011) and it could be possible that this animal response varies between multiparous and primiparous cows. Therefore, it remains a possibility that the difference in DMI between the control and the cooling periods could be due to extreme variation in intake in one of the parity groups but not in the other. Similarly, HS and changes in DMI can have effects on milk composition (Wheelock *et al*. 2010); however, this was not considered during the current trial and could have helped better identify changes in the carbohydrate and/or protein metabolism. These aspects must be kept in mind when further interpreting the current results and future research is needed to confirm our assumptions. Additionally, because of the belief that cows radiate heat at night, due to the cooler temperatures, cooling is normally restricted to the hottest hours of the day. Nevertheless, HS alleviation may be needed also at night or for an extended period during the day, a strategy that deserves further attention.

Rainy season versus dry season

As mentioned above, clear differences appeared in environmental parameters between the dry and the rainy season. The THI during the rainy season was, on average, 2 units greater than that in the dry season, mainly as the result of a much higher RH in that period. In theory, the higher THI would have consequences on MY. For example, Ravagnolo and Misztal (2000) stated that for every unit increase in THI beyond 72, where the onset of HS was found in that study, MY declined by 0.2 kg, but a similar response was not observed in the current study when comparing both seasons. Animals during the rainy season had a slightly higher respiration rate than they had during the dry season (73.8 vs 68.3 breaths/min respectively), but rectal temperature remained unaffected (39.0°C and 39.2°C for rainy and dry seasons respectively). Cows utilise panting as a mechanism to release heat and avoid body temperature increments, and, together with other metabolic and insulative adaptations, this could have been sufficient to avoid decreases in production driven by HS, as suggested, for example, by Webster *et al*. (1975). Additionally, it is possible that animals modified their behaviour towards generating less heat, including decreasing feed intake, during the rainy season, factors that were not accounted for in the current study and should be considered in future trials.

The THI is driven by different weather variables in the rainy season from those in the dry season. During the dry season, AT is the most prominent driver of THI, whereas during the rainy season RH is the main contributor to it. The different environmental factors may cause different responses by the animal, as stated by McDowell *et al*. (1976), particularly in the tropics, where at maximum AT, RH is at a minimum and *vice versa* (Kabuga 1992), and as confirmed in the current study (Fig. 2*a*). The inverse relationship between AT and RH was confirmed also in their effects on rectal temperature and respiration rate, with RH appearing to decrease rectal temperature in the rainy season compared with the dry season, whereas AT increased rectal temperature of primiparous and multiparous cows in the rainy season compared with the dry season. Respiration rate was more uniformly affected by AT and RH and was higher during the rainy season and for primiparous cows. When comparing the F statistic of the regressions in Table 3 for RH, AT or THI, the latter had the highest F statistic for both rectal temperature and respiration rate (6.84 and 8.80 respectively) compared with AT (5.05 and 7.51 respectively) and RH (1.38 and 0.99 respectively), indicating that THI was still the better predictor of both rectal temperature and respiration rate, but also highlighting that AT, and not RH, is the main driver of those responses.

Conclusions

The current study showed that, in contrast with common knowledge but in agreement with our first hypothesis, primiparous cows were more susceptible to the effects of HS than were multiparous cows; this was likely to be due to the smaller differences found in production and body size than the differences found between primiparous and multiparous high-yielding cows, from which most of the understanding on HS derives from. Because of this, primiparous cows benefitted the most from a cooling treatment. There was evidence that animals continued suffering HS during the times of the day without cooling, suggesting that adjustments in the cooling protocol can generate greater benefits. Interestingly, and in contrast to our second hypothesis, there were no changes in FCE between the animals with and those without cooling treatment, but a more accurate measurement of intake is needed to confirm this observation. There were no differences in the effects of HS on cows during the rainy or the dry season despite the higher THI in the former, which rejected our third hypothesis. An increased respiration rate during the rainy season was identified as one of the main mechanisms by which cows successfully regulate their body temperature; however, the cows could have made other behavioural adaptations towards decreasing heat production, including reducing feed intake, which was not measured during the rainy season. The effects observed in the current study, which in some cases contrast with available information from herds in the northern hemisphere, are likely to be due to the combination of several factors, such as breed, environmental conditions, smaller cow size, lower level of production or intake. Nevertheless, the current results certainly highlighted the need for further understanding on the effects of HS in lactating cows under hot climates.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

The authors acknowledge funding of the study from project ELS 5011 of the International Atomic Energy Agency (IAEA), and are very thankful to the Astoria de RL Cooperative for the use of their animals and facilities as well as field assistance during the execution of the current trial. The work of students Fatima Gonzalez, Alfonso Mendoza and Leonel Linares during the data collection is also acknowledged.

References

Aguilar I, Misztal I, Tsuruta S (2009) Genetic components of heat stress for dairy cattle with multiple lactations. *Journal of Dairy Science* **92**, 5702–5711. doi:10.3168/jds.2008-1928

Aguilar I, Misztal I, Tsuruta S (2010) Genetic trends of milk yield under heat stress for US Holsteins. *Journal of Dairy Science* **93**, 1754–1758. doi:10.3168/jds.2009-2756

Ahmed BMS, Younas U, Asar TO, Dikmen S, Hansen PJ, Dahl GE (2017) Cows exposed to heat stress during fetal life exhibit improved thermal tolerance. *Journal of Animal Science* **95**, 3497–3503. doi:10.2527/jas2016.1298

AOAC International (2005) 'Official methods of analysis.' 18th edn. (AOAC International: Gaithersburg, MD, USA)

Armstrong DV (1994) Heat stress interactions with shade and cooling. *Journal of Dairy Science* **77**, 2044–2050. doi:10.3168/jds.S0022-0302(94)77149-6

Baumgard LH, Rhoads RP (2012) Ruminant Nutrition Symposium: ruminant production and metabolic responses to heat stress. *Journal of Animal Science* **90**, 1855–1865. doi:10.2527/jas.2011-4675

Baumgard L, Wheelock J, Sanders S, Moore C, Green H, Waldron M, Rhoads R (2011) Postabsorptive carbohydrate adaptations to heat stress and monensin supplementation in lactating Holstein cows. *Journal of Dairy Science* **94**, 5620–5633. doi:10.3168/jds.2011-4462

Berman A (2011) Invited review: are adaptations present to support dairy cattle productivity in warm climates? *Journal of Dairy Science* **94**, 2147–2158. doi:10.3168/jds.2010-3962

Bernabucci U, Biffani S, Buggiotti L, Vitali A, Lacetera N, Nardone A (2014) The effects of heat stress in Italian Holstein dairy cattle. *Journal of Dairy Science* **97**, 471–486. doi:10.3168/jds.2013-6611

Colditz PJ, Kellaway RC (1972) The effect of diet and heat stress on feed intake, growth and nitrogen metabolism in friesian F1 brahman X friesian, and brahman heifers. *Australian Journal of Agricultural Research* **23**, 717–725.

Cowley F, Barber D, Houlihan A, Poppi D (2015) Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism. *Journal of Dairy Science* **98**, 2356–2368. doi:10.3168/jds.2014-8442

Finch VA (1986) Body temperature in beef cattle: its control and relevance to production in the tropics. *Journal of Animal Science* **62**, 531–542. doi:10.2527/jas1986.622531x

Flamenbaum I, Wolfenson D, Maman M, Berman A (1986) Cooling dairy cattle by a combination of sprinkling and forced ventilation and its implementation in the shelter system. *Journal of Dairy Science* **69**, 3140–3147. doi:10.3168/jds.S0022-0302(86)80778-0

Hahn GL (1999) Dynamic responses of cattle to thermal heat loads. *Journal of Animal Science* **77**(Suppl 2), 10–20. doi:10.2527/1997.77suppl_210x

Kabuga JD (1992) The influence of thermal conditions on rectal temperature, respiration rate and pulse rate of lactating Holstein–Friesian cows in the humid tropics. *International Journal of Biometeorology* **36**, 146–150. doi:10.1007/BF01224817

Lees AM, Sejian V, Wallage AL, Steel CC, Mader TL, Lees JC, Gaughan JB (2019) The impact of heat load on cattle. *Animals* **9**, 322. doi:10.3390/ani9060322

Lohrenz AK, Duske K, Schneider F, Nürnberg K, Losand B, Seyfert HM, Metges CC, Hammon HM (2010) Milk performance and glucose metabolism in dairy cows fed rumen-protected fat during mid lactation. *Journal of Dairy Science* **93**, 5867–5876. doi:10.3168/jds.2010-3342

McDowell RE, Hooven NW, Camoens JK (1976) Effect of climate on performance of Holsteins in first lactation. *Journal of Dairy Science* **59**, 965–971. doi:10.3168/jds.S0022-0302(76)84305-6

Min L, Zhao S, Tian H, Zhou X, Zhang Y, Li S, Yang H, Zheng N, Wang J (2017) Metabolic responses and 'omics' technologies for elucidating the effects of heat stress in dairy cows. *International Journal of Biometeorology* **61**, 1149–1158. doi:10.1007/s00484-016-1283-z

Ministry of Environment and Natural Resources (2015) (Ministerio de Medio Ambiente y Recursos Naturales). 'Annual meteorological bulletin.' (Meteorological Services Division: Santa Tecla, El Salvador)

Moran J (2005) 'Tropical dairy farming: feeding management for small holder dairy farmers in the humid tropics.' (CSIRO Publishing, Landlinks Press: Melbourne, Vic., Australia)

Neave HW, Lomb J, von Keyserlingk MAG, Behnam-Shabahang A, Weary DM (2017) Parity differences in the behavior of transition dairy cows. *Journal of Dairy Science* **100**, 548–561. doi:10.3168/jds.2016-10987

Poncheki JK, Schultz ML, Viechnieski SL, Almeida R (2015) Analysis of daily body weight of dairy cows in early lactation and associations with productive and reproductive performance. *Revista Brasileira de Zootecnia* **44**, 187–192. doi:10.1590/S1806-92902015000500004

Ravagnolo O, Misztal I (2000) Genetic component of heat stress in dairy cattle, parameter estimation. *Journal of Dairy Science* **83**, 2126–2130. doi:10.3168/jds.S0022-0302(00)75095-8

Ribeiro N, Costa R, Filho E, Ribeiro M, Bozzi R (2018) Effects of the dry and the rainy season on endocrine and physiologic profiles of goats in the Brazilian semi-arid region. *Italian Journal of Animal Science* **17**, 454–461. doi:10.1080/1828051X.2017.1393320

Society of the Physiology of Nutrition (2011) (Gesselschaft fuer Ernaehrungsphysiologie). 'Recommendations for energy and nutrients requirements for dairy cattle and heifers. No. 8.' (DLG–Verlags–GmbH: Frankfurt, Germany) [In German]

Streffer C (1988) Aspects of metabolic change after hyperthermia. In 'Application of hyperthermia in the treatment of cancer. Recent results in cancer research, vol 107'. (Eds RD Issels, W Wilmanns) pp. 7–16. (Springer: Berlin, Germany)

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2

Wathes DC, Cheng Z, Bourne N, Taylor VJ, Coffey MP, Brotherstone S (2007) Differences between primiparous and multiparous dairy cows in the inter-relationships between metabolic traits, milk yield and body condition score in the periparturient period. *Domestic Animal Endocrinology* **33**, 203–225. doi:10.1016/j.domaniend.2006.05.004

Webster AJF, Osuji PO, White F, Ingram JF (1975) The influence of food intake on portal blood flow and heat production in the digestive tract of sheep. *British Journal of Nutrition* **34**, 125–139. doi:10.1017/S0007114575000165

West JW (2003) Effects of heat-stress on production in dairy cattle. *Journal of Dairy Science* **86**, 2131–2144. doi:10.3168/jds.S0022-0302(03)73803-X

Wheelock JB, Rhoads RP, Van Baale MJ, Sanders SR, Baumgard LH (2010) Effects of heat stress on energetic metabolism in lactating Holstein cows. *Journal of Dairy Science* **93**, 644–655. doi:10.3168/jds.2009-2295

Handling editor: John Gaughan