Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin¹

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ABSTRACT

Heat stress is detrimental to dairy production and affects numerous variables including feed intake and milk production. It is unclear, however, whether decreased milk yield is primarily due to the associated reduction in feed intake or the cumulative effects of heat stress on feed intake, metabolism, and physiology of dairy cattle. To distinguish between direct (not mediated by feed intake) and indirect (mediated by feed intake) effects of heat stress on physiological and metabolic indices, Holstein cows ($n = 6$) housed in thermal neutral conditions were pair-fed (PF) to match the nutrient intake of heat-stressed cows (HS; $n = 6$). All cows were subjected to 2 experimental periods: 1) thermal neutral and ad libitum intake for 9 d (P1) and 2) HS or PF for 9 d (P2). Heat-stress conditions were cyclical with daily temperatures ranging from 29.7 to 39.2°C. During P1 and P2 all cows received i.v. challenges of epinephrine (d 6 of each period), and growth hormone releasing factor (GRF; d 7 of each period), and had circulating somatotropin (ST) profiles characterized (every 15 min for 6 h on d 8 of each period). During P2, HS cows were hyperthermic for the entire day and peak differences in rectal temperatures and respiration rates occurred in the afternoon (38.7 to 40.2°C and 46 to 82 breaths/min, respectively). Heat stress decreased dry matter intake by greater than 35% and, by design, PF cows had similar reduced intakes. Heat stress and PF decreased milk yield, although the pattern and magnitude (40 and 21%, respectively) differed between treatments. The reduction in dry matter intake caused by HS accounted for only approximately 35% of the

decrease in milk production. Both HS and PF cows entered into negative energy balance, but only PF cows had increased (approximately 120%) basal nonesterified fatty acid (NEFA) concentrations. Both PF and HS cows had decreased (7%) plasma glucose levels. The NEFA response to epinephrine did not differ between treatments but was increased (greater than 50%) in all cows during P2. During P2, HS (but not PF) cows had a modest reduction (16%) in plasma insulin-like growth factor-I. Neither treatment nor period had an effect on the ST response to GRF and there was little or no treatment effect on mean ST levels or pulsatility characteristics, but both HS and PF cows had reduced mean ST concentrations during P2. In summary, reduced nutrient intake accounted for just 35% of the HS-induced decrease in milk yield, and modest changes in the somatotrophic axis may have contributed to a portion of the remainder. Differences in basal NEFA between PF and HS cows suggest a shift in postabsorptive metabolism and nutrient partitioning that may explain the additional reduction in milk yield in cows experiencing a thermal load.

Key words: heat stress, somatotropin, metabolism, hyperthermia

INTRODUCTION

Animal productivity and efficiency are significantly influenced by environmental factors. Ambient temperatures above the thermal neutral zone are detrimental to lactation, growth, and reproduction in all agriculturally important species, but the effects on the dairy industry are the most economically severe (St. Pierre et al., 2003). The reduction in revenue associated with heat stress is not only a result of decreased milk yield, but also includes impaired reproduction, increased health care costs, and reduced milk quality. In addition, heat stress reduces feed efficiency and nutrient utilization, but the biological mechanism(s) mediating this altered nutrient partitioning are not well understood.

Heat stress markedly reduces DMI and milk yield and because the decreased DMI precedes the reduc-

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tion in milk production, it is generally accepted that reduced nutrient intake is primarily responsible for the diminished milk synthesis (see reviews by Fuquay, 1981; Beede and Collier, 1986; West, 2003). However, a thermal load may directly affect milk yield by unknown mechanisms that are independent of reduced DMI. For example, heat stress up-regulates a variety of protein chaperone genes and interferes with cytoskeletal and cell transport function in bovine mammary epithelial cells *in vitro* (Collier et al., 2008) but how this alters milk synthesizing machinery *in vivo* is not clear. Furthermore, we recently demonstrated that heat-stressed cows do not display the typical metabolic profile (i.e., increased NEFA levels) of an animal on a lowered plane of nutrition (Shwartz et al., 2009). Consequently, it appears that heat stress affects cellular physiology and systemic metabolism, but it is difficult to differentiate between the direct versus indirect (mediated by reduced feed intake) effects of a thermal load.

Milk synthesis and secretion are complicated processes that are governed by several hormones and are sensitive to both physiological and environmental cues. Some of the most potent and well-characterized lactogenic hormones are members of the somatotrophic axis: somatotropin (**ST**) and IGF-I (Bauman and Vernon, 1993). During positive energy balance (**EBAL**), pituitary-derived ST partitions nutrients toward the mammary gland by decreasing the nutrient uptake of extra-mammary tissues and stimulating hepatic IGF-I synthesis and secretion. The reduced nutrient uptake by adipose and muscle tissues coupled with increased IGF-I (a potent stimulator of milk synthesis) are examples of the direct and indirect mechanisms, respectively, by which recombinant (**r**)bST increases milk yield (Bauman, 1999). However, during negative EBAL, the somatotrophic axis uncouples and hepatic IGF production decreases despite increased circulating ST concentrations (McGuire et al., 1992a). Thus, during times of somatotrophic axis uncoupling, ST remains galactopoietic via partitioning nutrients (dietary and tissue derived) toward milk synthesis.

Interestingly, the negative EBAL caused by heat stress and early lactation appear to differentially affect the somatotrophic axis. For example, heat-stressed cows in a calculated negative EBAL (Moore et al., 2005; Shwartz et al., 2009) have or tend to have reduced ST levels (Mohammed and Johnson, 1985; Igono et al., 1988; McGuire et al., 1991). The lack of an increase in both ST and NEFA levels during heat stress suggests an altered role for the somatotrophic axis in mediating the cows' physiological response to heat stress. Therefore, study objectives were to distinguish the direct effects of heat stress from the indirect effects (mediated by

reduced DMI) by comparing production parameters, metabolism, and selected components of the somatotrophic axis in lactating Holstein cows.

MATERIALS AND METHODS

Animals

Twelve multiparous (2.4 ± 0.5 parity), lactating Holstein cows (140 ± 13 DIM, 663 ± 68 kg of BW) were randomly assigned to individual tie stalls in 1 of 2 environmental chambers at the William J. Parker Agricultural Research Complex (Tucson, AZ). Throughout the experiment, cows were milked twice daily (0500 and 1700 h) and milk yields were recorded at each milking. All cows were individually fed a TMR twice daily (0500 and 1700 h) and orts were recorded daily before the morning feeding. The TMR was formulated by Dairy Nutrition Services (Chandler, AZ) to meet or exceed the predicted requirements (NRC, 2001) of energy, protein, minerals, and vitamins (Table 1). Alfalfa hay was the primary forage with steam-flaked corn as the primary concentrate. The TMR was sampled weekly and analyzed by wet chemistry methods (Chandler, AZ). All procedures were reviewed and approved by the University of Arizona Institutional Animal Care and Use Committee.

Experimental Design

After adjusting to the environmental chambers (7 d), cows in both treatment groups were exposed to constant thermal neutral conditions [20°C , 20% humidity; temperature-humidity index (**THI**) = 64; 12-h light and dark cycles] and allowed to eat *ad libitum* for 9 d [experimental period (**P**) 1]. Period 1 and P2 were separated by 7 d, and cows remained in thermal neutral conditions between periods. During P2 (9 d) cows in group 1 remained in the same thermal neutral conditions while cows in group 2 were heat-stressed (**HS**). The HS cows endured cyclical temperatures (to mimic daily variation) ranging from 29.4 to 38.9°C with constant 20% humidity and 12-h light and dark cycles and were fed *ad libitum*. Between 0000 and 0700 h, the THI remained at 73; thereafter, the conditions became increasingly warmer until peaking at a THI of 82 between 1300 and 1500 h. After peak THI, temperatures gradually declined until the THI again reached 73 at 2300 h. Reductions in daily feed intake by HS cows in P2 were determined as a percentage of their mean daily *ad libitum* intake in P1. Cows in group 1 were pair-fed (**PF**) during P2 to match the percentage reduction in intake of the HS cows.

Thermal Status Measurements

Body temperature indices (respiration rate, skin and rectal temperatures) were obtained 4 times daily (0600, 1000, 1400, and 1800 h). Respiration rates were determined by counting flank movements for 60 s. Skin temperatures were measured on a shaved patch (approximately 5 cm²) on the shoulder with an infrared temperature gun (Raynger MX model RayMX4PU, Raytek, Santa Cruz, CA; accuracy $\pm 1.0^{\circ}\text{C}$). Rectal temperatures were measured using a standard digital thermometer (GLA M700 Digital Thermometer, GLA Agricultural Electronics, San Luis Obispo, CA; accuracy $\pm 0.1^{\circ}\text{C}$).

Milk and Blood Sampling

Milk samples from each cow (from both the morning and evening milking) were collected on d 1, 3, 6, and 9 of each period and stored at 4°C with a preservative (bronopol tablet, D&F Control System, San Ramon, CA) until analysis by Arizona DHIA (Tempe, AZ) using AOAC (2000)-approved infrared analysis equipment and procedures for milk components.

Blood samples were collected daily during both periods by coccygeal venipuncture into evacuated glass tubes containing 250 units of sodium heparin (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Blood samples remained on ice until plasma was harvested following centrifugation at $1,500 \times g$ for 15 min and subsequently frozen at -20°C until analysis.

Net EBAL Analysis

Body weights were obtained on all animals on d 1, 4, and 9 of each period and were used to calculate net EBAL using the following equation: $\text{EBAL} = \text{net energy intake} - (\text{NE}_M + \text{NE}_L)$. Maintenance energy requirement was calculated using the equation (NRC, 2001): $\text{NE}_M = (0.08 \times \text{BW}^{0.75})$ and was increased by 25% during the HS conditions as recommended (NRC, 1989). Net energy for lactation was calculated as $\text{NE}_L = [(0.0929 \times \text{fat } \%) + (0.0547 \times \text{CP } \%) + (0.0395 \times \text{Lactose } \%)] \times \text{milk yield}$ (NRC, 2001).

Epinephrine Challenge

Bilateral indwelling jugular catheters were inserted into all cows on d 4 of each period. Epinephrine challenges (1.4 $\mu\text{g/kg}$ of BW) were administered as described previously by Baumgard et al. (2002a) on d 6 of each period at 0800 h, immediately following the morning milking and feeding. Epinephrine HCl (1 mg/mL, Anpro Pharmaceutical, Arcadia, CA) was administered

Table 1. Ingredients and chemical composition of diets¹

Item	% of DM
Ingredient	
Alfalfa hay	51.7
Steam-flaked corn	19.2
Whole cottonseed	9.3
Amino plus ²	5.4
Dried citrus pulp	5.4
Molasses	4.6
Supplement ³	2.5
Maxxer ⁴	1.9
Chemical analysis, % of DM	
CP	17.2
NDF	36.5
ADF	27.9
NE _L , Mcal/kg of DM	1.77

¹Values represent an average of samples collected and composited throughout the trial. Diet DM averaged 49.4%.

²Soybean-based supplement; 51.7% CP (Ag. Processing Corp., Hastings, NE).

³The supplement contained 1.14% fat, 10.42% Ca, 4.49% P, 3.80% Mg, 0.49% S, 0.19% K, 15.83% Na, 7.52% Cl, 2,029.06 mg/kg of Zn, 1,991.82 mg/kg of Mn, 974.24 mg/kg of Fe, 583.45 mg/kg of Cu, 67.86 mg/kg of Co, 12.28 mg/kg Se, 6.81 mg/kg of Mo, 43.68 mg/kg of I, 304.9 of IU/g of vitamin A, 30.2 IU/g of vitamin D, and 1.0 IU/g of vitamin E.

⁴Calcium salts of palm oil (Tarome Inc., Eloy, AZ).

via the jugular catheter and immediately chased with 12 mL of sterile saline. Blood samples were collected at -30 , -20 , -10 , 0 , 2.5 , 5 , 7.5 , 10 , 20 , 30 , 45 , 60 , 90 , and 120 min relative to epinephrine administration. Samples were collected by syringe into disposable glass culture tubes containing 250 units of sodium heparin and immediately placed on ice. After centrifugation, plasma was divided into 2 aliquots, which were both frozen at -20°C for subsequent analysis of plasma NEFA concentration.

Growth Hormone Releasing Factor Challenge

Growth hormone releasing factor (GRF) challenges were administered via the jugular catheter on d 7 of each period using a modification of the procedure described by Baumgard et al. (2002b). A human GRF analog (stock solution: 10 $\mu\text{g/mL}$ in sterile physiological saline containing 0.1% BSA) was prepared and a "clearing" GRF challenge (5 $\mu\text{g/100 kg}$ of BW) administered at 0800 h (after the morning milking and feeding). Blood samples were collected at -30 , -20 , -10 , 0 , 2.5 , 5 , 7.5 , 20 , 30 , 45 , 60 , and 90 min relative to GRF administration. At 120 min after the initial GRF administration, a second GRF challenge (5 $\mu\text{g/100 kg}$ of BW) was administered and blood samples collected as described for the clearing challenge. Blood samples were collected by syringe from the jugular catheters into disposable glass culture tubes containing 250 units of sodium heparin

and immediately placed on ice. After centrifugation, plasma was divided into 2 aliquots, which were both frozen at -20°C for later analysis of plasma ST concentrations.

Somatotropin Pulsatility

A 6-h window bleed was conducted on all cows on d 8 of both periods starting at 0800 h. Blood samples were collected from indwelling jugular vein catheters at 15-min intervals. Samples were collected into disposable glass culture tubes containing 250 units of sodium heparin and immediately placed on ice. After centrifugation, plasma was divided into 2 aliquots which were both frozen at -20°C for subsequent analysis of plasma ST concentration.

Plasma Analyses

All plasma NEFA and glucose concentrations were measured enzymatically using commercially available kits validated in our laboratory (NEFA C kit and Autokit Glucose C2, Wako Chemicals USA, Richmond, VA). The intra- and interassay coefficients of variation (CV) were 2.9 and 2.4% for glucose and 8.8 and 7.9% for NEFA, respectively. Plasma ST and IGF-I concentrations were determined in triplicate by radioimmunoassay as described previously (Weber et al., 2007). The minimal detectable concentration of ST was 0.7 ng/mL of standard or sample added to the assay tubes. Intra- and interassay CV were 3.1 and 6.8%, respectively. The minimal detectable concentration of IGF-I was 0.2 ng/mL of standard or sample added to the assay tubes. Intra- and interassay CV were 2.7 and 3.3%, respectively.

Calculations and Statistical Analyses

The NEFA and ST response to epinephrine and GRF were calculated as area under the curve (AUC) by use of linear trapezoidal summation between successive pairs of NEFA or ST concentrations and time coordinates after correcting for the mean baseline levels (Baumgard et al., 2002a,b). Baseline concentrations were defined as the mean of the 3 samples before epinephrine or GRF administration. The AUC was determined for 30-, 45-, 60-, and 90-min intervals after epinephrine or GRF administration, and statistical and biological interpretation of these results did not differ among the intervals. The NEFA AUC was reported from zero to 45 min and ST AUC reported from zero to 60 min after epinephrine and GRF administration, respectively. Plasma ST concentration profile characteristics (pulsatility) were determined using CLUSTER8 (ver. 1, 2004; [\[mljohnson.pharm.virginia.edu\]\(http://mljohnson.pharm.virginia.edu\)\) as originally described \(Veldhuis and Johnson, 1986\). A \$2 \times 1\$ cluster configuration with a t-statistic of 2.0 was used to determine mean ST concentration, number and duration of peaks and valleys, peak height, peak area, valley mean and nadir.](http://</p>
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Effects of group, period, and their interaction were assessed as a completely randomized design using PROC MIXED (SAS Institute, 2005). A repeated measures analysis with an autoregressive covariance structure and day as the repeated effect was used to determine effects of day, group, and their interaction on DMI, milk yield, and milk composition during P1. A covariate (mean value per cow for each variable during period 1) was added to the model to determine effects of day, group, and their interaction during P2. Results are reported as least squares means, and means were considered to differ when $P \leq 0.05$ and to tend to differ if $P < 0.10$.

RESULTS

During P1, all cows were managed the same regardless of their assigned treatment and were housed in a thermal neutral climate (identical ambient temperature and humidity) and allowed to eat ad libitum. In P1, body temperature indices were similar for both PF and HS cows, except at 1800 h when surface temperatures were slightly lower ($P < 0.05$) and rectal temperatures were slightly higher ($P < 0.05$) for HS cows (Table 2). There was a treatment by period interaction ($P < 0.01$) for surface temperature and respiration rate as these values were lower ($P < 0.05$) or tended ($P < 0.10$) to decrease in the PF cows but increased in the HS cows (Table 2) during P2. The maximum rectal and surface temperature differences (1.90 and 4.95°C) between treatments occurred at 1800 h, whereas the biggest difference in respiration rate (51 breaths/min) occurred at 1400 h. Interestingly, surface temperatures and respiration rates (but not rectal temperatures) either numerically or statistically decreased (an average of 0.99°C and 5.6 breaths/min) in the PF group (P1 vs. P2; Table 2).

Dry matter intake was greater ($P < 0.01$) for group 2 than group 1 cows in both periods. During P2, DMI progressively declined in HS cows (and by design PF cows had a similar pattern) for the first 4 to 5 d before feed intake stabilized (an average decrease of 7.45 kg/d; Figure 1A). Heat stress decreased DMI ($P < 0.01$) by 37% by d 9 (Figure 1A; Table 3). Heat stress and PF decreased ($P < 0.01$) milk yield 40.5 and 20.9% (nadir), respectively, compared with P1 (Figure 1B). Cows experiencing HS produced approximately 7.5 kg/d less ($P < 0.01$) milk than PF cows. During P2, both

Table 2. Effects of heat stress (HS) or pair-feeding (PF) on body temperature indices in lactating Holstein cows

Parameter	Period 1 ¹		Period 2 ²		SEM	P-value		
	Group 1 (TN)	Group 2 (TN)	Group 1 (PF)	Group 2 (HS)		Group	PER	Group × PER
Surface, ³ °C								
0600 h	33.37 ^a	33.35 ^a	31.30 ^b	35.41 ^c	0.16	<0.01	0.95	<0.01
1000 h	34.05 ^a	33.70 ^a	33.07 ^b	36.61 ^c	0.13	<0.01	<0.01	<0.01
1400 h	33.82 ^a	33.54 ^a	33.72 ^a	38.04 ^c	0.12	<0.01	<0.01	<0.01
1800 h	34.00 ^a	33.55 ^b	33.21 ^b	38.16 ^c	0.14	<0.01	<0.01	<0.01
Rectal, °C								
0600 h	38.40 ^{ab}	38.54 ^a	38.30 ^b	39.25 ^c	0.06	<0.01	<0.01	<0.01
1000 h	38.42 ^a	38.53 ^a	38.50 ^a	39.28 ^b	0.06	<0.01	<0.01	<0.01
1400 h	38.50 ^a	38.59 ^a	38.51 ^a	40.12 ^b	0.06	<0.01	<0.01	<0.01
1800 h	38.43 ^a	38.62 ^b	38.48 ^{ab}	40.38 ^c	0.05	<0.01	<0.01	<0.01
Respiration, breaths/min								
0600 h	36.3 ^a	38.4 ^a	29.5 ^b	57.5 ^c	1.0	<0.01	<0.01	<0.01
1000 h	38.3 ^a	40.6 ^a	30.6 ^b	62.7 ^c	1.4	<0.01	<0.01	<0.01
1400 h	39.9 ^a	41.1 ^a	33.4 ^b	84.6 ^c	1.4	<0.01	<0.01	<0.01
1800 h	39.8 ^{ab}	42.7 ^a	38.4 ^b	77.5 ^c	1.4	<0.01	<0.01	<0.01

^{a-c}Values within row of each variable with differing superscripts indicate statistical difference.

¹During period 1, cows in both groups were treated identically [housed in thermal neutral (TN) conditions and allowed to eat ad libitum].

²During period 2, cows were heat-stressed and allowed to eat ad libitum or pair-fed and kept in TN conditions.

³Obtained at the shoulder.

PF and HS decreased ($P < 0.03$) milk lactose content (4.75 in P1 vs. 4.64% in P2; Table 3; Figure 2A). Milk fat did not differ between environmental treatments, but increased from P1 to P2 (3.53 vs. 3.79%; Table 3; Figure 2B). There tended to be a treatment × period interaction on milk protein as it remained stable in PF cows but decreased when cows were exposed to HS ($P < 0.09$; Table 3; Figure 2C).

Cows that were heat-stressed had higher EBAL values in both periods ($P < 0.01$), but both HS and PF cows had markedly reduced EBAL during P2 compared with P1 ($P < 0.01$; Table 3). During P2 both PF and HS cows lost a similar quantity of BW ($P > 0.35$; 49.4 kg; data not shown). Plasma glucose concentrations were greater in PF cows during both periods ($P < 0.05$) and were reduced ($P < 0.01$) when intake declined during P2. Plasma NEFA concentrations were similar between treatments during P1. Daily plasma NEFA concentrations were not altered by HS but were increased (2×; $P < 0.01$; Table 3) in PF cows. Circulating NEFA concentrations during the P1 6-h window bleed did not differ between treatments, but were markedly increased during P2 in the PF compared with HS cows (506 and 149 $\mu\text{Eq/L}$, respectively; data not shown). The NEFA response to the epinephrine challenge tended to be greater ($P < 0.08$) in cows destined for heat stress during P1 and this difference remained during P2 (Table 4). Compared with P1, both PF and HS cows had larger (52%; $P < 0.01$) NEFA responses to the lipolytic stimuli during P2.

Environment had no effect on basal ST before the GRF administration or on ST response to the first (clearing dose) or second GRF challenge (Table 4). During the 6-h window bleed, HS cows tended ($P < 0.07$) to have reduced circulating average ST concentrations and this difference existed in both P1 and P2 (Table 5). The number of ST peaks (secretion events) and most of the ST pulse characteristics were unaffected by treatment, period, or their interaction (Table 5). The mean peak height, peak area, mean valley ST concentration and valley nadir either tended ($P < 0.09$) to be or were lower ($P < 0.05$) in HS cows during both periods. Both PF and HS reduced ($P < 0.05$) mean circulating ST concentration, valley ST concentration, and mean valley ST nadir (Table 5). Heat-stressed cows had slightly greater plasma IGF-I levels (13%; $P < 0.05$) than PF cows in P1 and this difference remained in P2. There was a treatment by day interaction ($P < 0.01$) during P2 as plasma IGF-I levels were modestly decreased (16%; $P < 0.05$) by the thermal load but were unaffected by plane of nutrition (Figure 3).

DISCUSSION

Thermal stress reduces animal productivity and decreases feed efficiency of agriculturally important species. Despite remarkable advances in construction design of animal housing facilities and cooling technology (Armstrong, 1994; Burgos et al., 2007), heat stress remains an incredibly costly issue for the dairy industry

Figure 1. Effects of heat stress (HS) or pair-feeding (PF) on A) DMI and B) milk yield in lactating Holstein cows. Solid lines with diamonds represent PF cows and dashed lines with squares represent HS cows. The mean value from d 1 to 9 of the thermal neutral ad libitum period (P1) was used as a covariate and is represented by P1 on the x-axis. The d 1 to 9 results are from P2 when cows were exposed to HS or exposed to thermal neutral conditions and PF with the HS cows.

(approximately \$900 million annually in the United States; St. Pierre et al., 2003). This figure may be grossly underestimated as the June 2006 heat wave is thought to have cost the California dairy industry \$1 billion (CDFA, 2006). The financial impact of environmental heat on the global dairy industry probably exceeds that of mastitis and other economically detrimental parameters (i.e., poor reproduction; N. St. Pierre, The Ohio State University, Columbus; personal communication) and this will certainly become more of an issue in the future as milk yield (and thus metabolic heat production) continues to increase. This clearly necessitates developing new strategies, likely based on physiological

or metabolic adaptations, that can help maintain milk yield during stressful summer months.

In the present study, exposure to an environmental heat load well above the bovine thermal comfort zone resulted in a marked increase of all body temperature variables measured. We chose to mimic a typical July day in Arizona and, although the room temperature dropped to 29.4°C during the night (for 8 h), HS cows still exhibited elevated rectal temperatures (0.95°C), surface temperatures (4.1°C), and increased respiration rate (28 breaths/min) at 0600 h indicating that the heat strain was maintained for the entire 24 h of each day. This level of heat strain was associated with

Table 3. Effects of pair-feeding (PF) or heat stress (HS) on production and metabolism variables in lactating Holstein cows

Parameter	Period 1 ¹		Period 2 ²		SEM	P-value		
	Group 1 (TN)	Group 2 (TN)	Group 1 (PF)	Group 2 (HS)		Group	PER	Group × PER
DMI, kg/d	21.2	23.4	15.6	16.8	0.4	<0.01	<0.01	0.27
Milk yield, kg/d	34.0 ^a	32.1 ^a	29.0 ^b	21.5 ^c	0.9	<0.01	<0.01	<0.01
Milk components								
Fat, %	3.50	3.56	3.68	3.90	0.09	0.14	<0.01	0.41
Protein, %	2.67 ^{ab}	2.73 ^a	2.69 ^{ab}	2.60 ^b	0.05	0.91	0.31	0.08
Lactose, %	4.77	4.72	4.68	4.60	0.04	0.11	0.02	0.79
SNF, %	8.26	8.27	8.19	8.02	0.09	0.40	0.10	0.33
EBAL, ³ Mcal/d	5.56	9.02	-1.27	2.03	0.53	<0.01	<0.01	0.88
Glucose, mg/dL	70.3	67.9	65.8	62.6	1.3	0.04	<0.01	0.76
NEFA, µEq/L	139 ^a	110 ^a	305 ^b	128 ^a	13	<0.01	<0.01	<0.01

^{a-c}Values within row of each variable with differing superscripts indicate statistical difference.

¹During period 1, cows in both PF and HS were treated identically [housed in thermal neutral (TN) conditions and allowed to eat ad libitum].

²During period 2, cows were heat-stressed and allowed to eat ad libitum or pair-fed and kept in TN conditions.

³Energy balance.

a precipitous decline in milk yield above what would have been predicted based on DMI (Figure 1A and 1B), indicating that reduced nutrient intake accounts for a minor portion of the lost milk production. Thus, factor(s) other than reduced feed intake are responsible for 64% of the milk loss differential.

The fact that the HS cows in the present study had decreased milk yield beyond that attributable to decreased feed intake contradicts the traditional dogma that heat-stress-induced reductions in milk yield are primarily a consequence of decreased nutrient intake (Wayman et al., 1962; Fuquay, 1981; Beede and Collier, 1986; West, 2003). During experiments similar to ours (i.e., separate heat stress and restricted intake groups) McGuire et al. (1989, 1991) determined that the heat-stress-induced reduction in feed intake was sufficient to explain most (if not all) of the reduction in milk yield. The key difference between the studies of McGuire et al. (1989, 1991) and the current study is the diurnal heat-load pattern. Specifically, their overnight temperature was 19°C and ours was 29°C. The marked reduction in evening ambient temperature allowed their cows to completely dissipate their heat load (based on morning rectal temperatures). Thus, the close association between DMI and milk production remained intact in the studies by McGuire et al. (1989, 1991). Our results, in agreement with previous data (Bianca, 1965), suggest that when overall heat stress (extent and duration) exceeds a given threshold (as yet unidentified) the cumulative thermal load disrupts the nutrient intake:milk production relationship and milk synthesis declines beyond expected levels. The factor(s) underlying this disruption must be identified so that nutritive, physiological, and management strategies can

be developed to combat the negative effects of severe thermal stress.

The disruption in the nutrient intake:milk production relationship described above emphasizes the importance of our experimental design because it enabled us to evaluate thermal stress while eliminating the confounding effects of dissimilar nutrient intake between thermal comfort and heat-stress conditions. Employing this type of approach is required to differentiate between the direct and indirect effects (e.g., reduced intake) of environment-induced hyperthermia because both heat-stressed and malnourished animals share common responses (i.e., reduced milk yield). This design is also critical in determining the extent to which an indirect effect of heat stress, such as reduced feed intake, plays a role in physiological or metabolic adaptations that occur during elevated environmental temperatures.

During P2, both PF and HS cows had a slight (2.1%) reduction in milk lactose concentrations (Figure 2A). The small heat-induced decrease in lactose agrees with our recent results (Shwartz et al., 2009) and previous reports (Nardone et al., 1997), but appears dependent upon reduced feed intake as PF cows had a similar temporal pattern. Milk fat content increased during P2 and was unaffected by treatment (Figure 2B). Although the increase in milk fat during heat stress agrees with a previous report (Regan and Richardson, 1938), it was slightly surprising as milk fat is typically depressed during the summer months on commercial dairies (Huber, 1996; Kadzere et al., 2002). Our recent data also demonstrate that climate-controlled HS cows do not have reduced milk fat concentrations (Shwartz et al., 2009) and together suggest that seasonal factors in addition to heat may contribute to reduced milk fat percentages

Figure 2. Effects of heat stress (HS) or pair-feeding (PF) on A) milk lactose content, B) milk fat content, and C) milk protein content in lactating Holstein cows. Solid lines with diamonds represent PF cows and dashed lines with squares represent HS cows. The mean value from d 1 to 9 of the thermal neutral ad libitum period (P1) was used as a covariate and is represented by P1 on the x-axis. The d 1 to 9 results are from P2 when cows were exposed to HS or exposed to thermal neutral conditions and PF with the HS cows.

during the summer. Our decreased milk protein concentration during heat stress agrees with the previous results (Regan and Richardson, 1938; Kadzere et al., 2002; Schwartz et al., 2009) and it appears that heat itself (rather than reduced amino acid intake) affects milk protein synthesizing machinery, especially α - and β -casein synthesis (Bernabucci et al., 2002).

We and others have demonstrated that a severe heat load causes lactating dairy cows to enter a period of negative EBAL (Moore et al., 2005; Settivari et al., 2007; Schwartz et al., 2009). Calculated EBAL for our PF and HS cows was reduced (6.83 and 6.99 Mcal/d,

respectively) but only the PF cows had a mean negative EBAL. Our previous climate-controlled study (Schwartz et al., 2009) indicated that HS cows entered into a more severe negative EBAL (about -4 Mcal/d), but HS cows in the current study had a more severe decrease in milk yield resulting in a slightly higher calculated energy balance. Calculating EBAL when an animal is experiencing a heat load requires increasing maintenance costs (McDowell et al., 1969; Morrison, 1983; Beede and Collier, 1986) and some estimate it to be greater than 30% (Fox and Tylutki, 1998). This assumes that there is a large energetic cost of dissipating stored heat and the NRC (1989) suggests using a correction factor of 1.25 when cows are severely heat-stressed. In addition, the Van't Hoff-Arrhenius temperature-coefficient equations predict a 20 to 30% increase in basal metabolic rate (and all chemical reactions) for each 1°C increase in body temperature (reviewed in Brody, 1945). Regardless of the accuracy of our calculated EBAL, both groups lost about 50 kg of BW during P2 and, by definition, animals losing BW are in a negative EBAL. However, we are unable to distinguish how much of the reduction in BW was due to mobilized tissue versus reduced gut fill (assumed because of reduced feed intake).

It is well established that fasting and severe malnutrition (energy or protein) result in a precipitous decline in circulating IGF-I concentrations and an increase in circulating ST levels in most species, including cattle (Phillips and Young, 1976; McGuire et al., 1992a; Thissen et al., 1994). This inverse relationship is referred to as an uncoupling of the ST-IGF axis and is caused in part by a reduction in ST-dependent hepatic IGF-I production. In contrast, results from our PF cows and from other studies (McGuire et al., 1992b) demonstrate that modest levels of undernutrition do not affect plasma IGF-I concentrations in lactating dairy cows. Therefore, the slight reductions in plasma IGF-I concentrations in our HS cows might indicate that the metabolic milieu favors uncoupling of the ST-IGF axis during heat stress. During circumstances in which the metabolic economy favors catabolism, ST-dependent hepatic IGF-I production is reduced and this uncoupling can be advantageous, such as during early lactation when substantial mobilization of body reserves is needed to meet lactation demands (Bauman and Currie 1980; Bell, 1995). The same may be true for HS animals but with a different objective as uncoupling of the axis reduces mammary nutrient utilization and favors nutrient use by peripheral tissues (i.e., skeletal muscle) to combat a thermal load rather than supporting anabolic processes. Regardless, 2 possible mechanisms may account for the HS-related decrease in plasma IGF-I concentration. First, the simplest explanation may be because of the corresponding reduction in circulating ST, as plasma

Table 4. Effects of pair-feeding (PF) or heat stress (HS) on the NEFA and somatotropin (ST) response to an epinephrine and growth hormone releasing factor (GRF) challenge in lactating Holstein cows

Parameter	Period 1 ¹		Period 2 ²		SEM	P-value		
	Group 1 (TN)	Group 2 (HS)	Group 1 (PF)	Group 2 (HS)		Group	PER	Group × PER
NEFA response								
AUC, ³ $\mu\text{Eq} \times \text{L} \times \text{min}$	3,866	2,660	5,342	4,554	535	0.08	<0.01	0.70
ST response to first GRF								
AUC, $\text{ng} \times \text{mL} \times \text{min}$	584	632	744	627	141	0.81	0.59	0.56
ST response to second GRF								
AUC, $\text{ng} \times \text{mL} \times \text{min}$	364	444	590	447	155	0.84	0.46	0.48

¹During period 1, cows in both PF and HS were treated identically [housed in thermal neutral (TN) conditions and allowed to eat ad libitum].

²During period 2, cows were heat-stressed and allowed to eat ad libitum or pair-fed and kept in TN conditions.

³Area under the curve.

IGF-I originates almost completely from the liver in an ST-dependent fashion (Sjogren et al., 1999; Yakar et al., 1999). However, a similar decline in circulating ST occurred without a corresponding reduction in plasma IGF-I in PF animals. A second mechanism may relate to the level of hepatic ST responsiveness in HS animals. In support of this notion, we recently demonstrated that HS cows increase hepatic IGF-I mRNA abundance in response to exogenous bST, albeit 33% less efficiently than PF cows (Wheelock et al., 2008). A recent report by Settivari et al. (2007) also demonstrates that HS cows remain responsive (in terms of plasma IGF-I) to exogenous bST, although it is unknown how this relates to a thermal neutral or pair-fed condition.

Previous chronic heat stress experiments indicate reduced circulating ST levels (Mohammed and Johnson, 1985; Igono et al., 1988; McGuire et al., 1991). However, capturing an accurate description of an animal's ST status requires multiple samples over time because of the pulsatile secretion pattern of ST. Our results indicate little or no effect of HS on ST pulsatility or pituitary responsiveness to GRF and this is not due to changes in systemic hydration as packed cell volume

does not change in this model (L. H. Baumgard and R. P. Rhoads; unpublished data). We anticipated that using a GRF clearing challenge would reduce the among-animal variation in response to the second GRF challenge and increase the opportunity to more accurately assess pituitary response to GRF (Suttie et al., 1991). However, results for both challenges were quite similar with no effect of HS or PF. The decreased ST values in HS cows agree with the aforementioned literature, but why the PF cows also had a decrease in ST (we anticipated it to increase) is not currently understood. Potential reasons include a small sample size (6 cows/treatment) and the fact that cows in neither treatment entered into severe negative EBAL.

During P2, PF and HS cows had reduced (7%) glucose concentrations and the progressive decrease over time (data not shown) closely resembles our previous climate-controlled heat stress research (Shwartz et al., 2009) and those of previous ruminant heat stress experiments (Achmadi et al., 1993; Itoh et al., 1998; Ronchi et al., 1999). Heat stress reduces total rumen VFA content (Schneider et al., 1988) and specifically the molar ratio of propionate:acetate (Kelley et al., 1966).

Table 5. Effects of pair-feeding (PF) or heat stress (HS) on somatotropin (ST) pulsatility characteristics in lactating Holstein cows

Parameter	Period 1 ¹		Period 2 ²		SEM	P-value		
	Group 1 (TN)	Group 2 (HS)	Group 1 (PF)	Group 2 (HS)		Group	PER	Group × PER
Mean ST, ng/mL	7.25	5.33	4.98	3.58	0.82	0.06	0.03	0.76
Peaks, n	3.7	3.4	3.8	4.0	0.3	0.87	0.21	0.47
Peak width, min	63	52	55	52	8	0.42	0.66	0.59
Peak mean height, ng/mL	9.56	6.08	7.22	4.84	1.0	0.01	0.10	0.60
Peak area, ng·min/mL	94	52	98	60	22	0.09	0.80	0.92
Valleys, n	4.0	4.2	4.0	4.7	0.4	0.24	0.53	0.53
Valley width, min	30	30	33	31	2	0.71	0.33	0.57
Valley mean ST, ng/mL	6.75	4.96	4.61	3.32	0.78	0.07	0.03	0.76
Valley ST nadir, ng/mL	6.29	4.49	3.94	2.90	0.75	0.08	0.02	0.63

¹During period 1, cows in both PF and HS were treated identically [housed in thermal neutral (TN) conditions and allowed to eat ad libitum].

²During period 2, cows were heat-stressed and allowed to eat ad libitum or pair-fed and kept in TN conditions.

Figure 3. Effects of heat stress (HS) or pair-feeding (PF) on plasma IGF-I levels in lactating Holstein cows. Solid lines with diamonds represent PF cows and dashed lines with squares represent HS cows. The mean value from d 1 to 9 of the thermal neutral ad libitum period (P1) was used as a covariate and is represented by P1 on the x-axis. The d 1 to 9 results are from P2 when cows were exposed to HS or exposed to thermal neutral conditions and PF with the HS cows. The standard error of the mean averaged 7.06 ng/mL.

However, the fact that PF cows had a similar glucose pattern suggests the decrease is probably the result of reduced hepatic propionate delivery, as propionate is the primary precursor in ruminant gluconeogenesis (Van Soest, 1982). The decrease in blood glucose may explain the small decrease in milk lactose content observed in both PF and HS cows.

During periods of nutritional insufficiency, adipose tissue lipolysis is enhanced and insulin-responsive tissues increase their use of NEFA as an energy source. This is one of several mechanisms that spares glucose for repartitioning to the mammary gland and helps maintain milk synthesis during periods of inadequate feed intake. In the current study, cows maintained on a lower plane of nutrition exhibited greater plasma NEFA levels both on a daily basis and during the 6-h window bleed (approximately 3.4-fold greater, data not shown) compared with the HS animals. The increased NEFA in PF cows was expected as plasma NEFA levels are thought to closely reflect calculated EBAL (Bauman et al., 1988). Heat-stressed cows did not appear to mobilize adipose tissue despite a loss of appetite and this agrees with our previous results (Shwartz et al., 2009), results of other heat-stressed ruminant studies (Sano et al., 1983; Itoh et al., 1998), and results from a malignant hyperthermic pig model (Hall et al., 1980). The lack of a NEFA increase in the HS cows is surprising as a thermal load (acute and chronic) increases blood

cortisol, epinephrine, and norepinephrine (Beede and Collier, 1986) and this hormonal profile normally results in lipolysis and adipose mobilization. Relative to when cows were fed ad libitum (P1), both the PF and HS cows had about a 2× greater NEFA response to the lipolytic signal. Their similar response to epinephrine during P2 coupled with their marked differences in basal NEFA concentrations indicates that hyperthermia does not increase adipocyte sensitivity like that of feed deprivation, but adipose tissue responsiveness to lipolytic signals remains similar to that of underfed cows. The mechanisms underlying these postabsorptive changes are currently unknown, but an increase in adipocyte fatty acid reesterification (predicted by the Van't Hoff-Arrhenius equation) and an increase in insulin action could explain the lack of basal adipose mobilization, because insulin is a potent lipolytic inhibitor (Vernon, 1992).

CONCLUSIONS

Identifying the mechanisms by which environmentally induced hyperthermia reduces production is a prerequisite for developing novel strategies to maintain milk yield during warm periods of the year and reduce the annual multibillion-dollar losses to the global dairy industry. This study provides data that clearly indicate that heat-induced reductions in nutrient intake can only

account for approximately 35% of the decrease in milk synthesis. Modest changes in the somatotrophic axis may explain a small portion of the reduction in milk yield during heat stress but such changes are unlikely to account for the remainder that is not explained by feed intake. An attractive hypothesis, partly based on the fact that heat-stressed cows do not appear to mobilize adipose tissue, is that heat stress causes metabolic adaptations preventing the enlistment of glucose-sparing mechanisms that normally prevent severe reductions in milk yield during periods of inadequate nutrient intake. It is currently unknown why heat-stressed cows reprioritize postabsorptive nutrient partitioning but identifying the mechanisms may provide clues on methods to ameliorate the negative effects of environment-related hyperthermia.

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