Effects of heat stress on energetic metabolism in lactating Holstein cows¹

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ABSTRACT

Heat stress has an enormous economic impact on the global dairy industry, but the mechanisms by which hyperthermia negatively affect systemic physiology and milk synthesis are not clear. Study objectives were to evaluate production parameters and metabolic variables in lactating dairy cows during short-term heat stress or pair-fed conditions coupled with bST administration. Twenty-two multiparous Holstein cows were subjected to 3 experimental periods: 1) thermoneutral conditions with ad libitum intake for 7 d (P1); 2) heat stress (HS) with ad libitum intake ($n = 10$) or pair-fed (PF) in thermoneutral conditions ($n = 12$) for 7 d (P2), and 3) 7 d of HS or PF in conditions as described in P2 with recombinant bovine somatotropin administered on d 1 (P3). All cows received an intravenous glucose tolerance test (GTT) on d 5 of each period. Heat stress conditions were cyclical and temperatures ranged from 29.4 to 38.9°C. Rectal temperatures and respiration rates increased during heat stress (38.6–40.4°C and 44–89 breaths/min, respectively). Heat stress reduced dry matter intake by 30% and by design PF cows had similar intake reductions (28%). During heat stress and pair-feeding, milk yield decreased by 27.6% (9.6 kg) and 13.9% (4.8 kg), respectively, indicating that reduced feed intake accounted for only 50% of the decreased milk production. Milk yield increased with recombinant bovine somatotropin in both HS (9.7%) and PF (16.1%) cows. Cows in both groups were in positive energy balance (3.95 Mcal/d) during P1 but entered negative energy balance during P2 and P3 (–5.65 Mcal/d). Heat stress and pair-feeding treatments decreased (9.3%) basal glucose concentrations. Heat stress conditions had no effect on basal NEFA levels during P2; however, PF cows (despite a similar calculated energy balance)

had a 2-fold increase in basal NEFA concentrations. Both groups had increased plasma urea nitrogen levels during P2 and P3 compared with P1. Basal insulin levels increased (37%) during P2 and P3 in HS cows but did not differ between periods in PF cows. During P2 and compared with P1, PF cows had a decreased rate of glucose disposal, whereas HS cows had a similar disposal rate following the GTT. During P2 and compared with P1, PF cows had a reduced insulin response whereas HS cows had a similar insulin response to the GTT. In summary, reduced nutrient intake accounted for only 50% of heat stress-induced decreases in milk yield, and feed intake-independent shifts in postabsorptive glucose and lipid homeostasis may contribute to the additional reduction in milk yield.

Key words: heat stress, insulin, metabolism, homeostasis

INTRODUCTION

Although advances in environmental cooling systems ameliorate production losses during summer months, heat stress continues to cost the American dairy industry approximately \$1 billion annually (St-Pierre et al., 2003). Factors contributing to this economic issue include decreased milk production, increased metabolic disorders and health problems (i.e., rumen acidosis, death), slow heifer growth, compromised milk quality, and reduced reproductive performance (Collier et al., 1982; West, 1999). Heat stress will become more of a concern in the future as metabolic heat production (i.e., body heat) increases in parallel with advancing milk yield and if climate change continues toward a warmer environment as some predict.

Reduced feed intake caused by heat stress has traditionally been assumed to be primarily responsible for the decrease in milk yield (Fuquay, 1981; Collier et al., 1982; West, 2003). However, we have recently demonstrated that reduced nutrient intake (indirect effects of heat) accounts for only about 35% of the heat stress-induced decrease in milk synthesis (Rhoads et al., 2009). A large portion of the direct effects (not mediated by decreased feed intake) of heat may be a consequence of energy intake-independent changes in nutrient partitioning. For instance, the metabolic profile of a heat-stressed cow differs from what would be expected from a lactat-

Received April 14, 2009.

Accepted September 26, 2009.

¹This work was partially funded by the United Dairymen of Arizona, The University of Arizona Experiment Station, #ARZT-136339-H-24-130, and the National Research Initiative Competitive Grant number 2008-35206-18817 from the USDA Cooperative State Research, Education, and Extension Service.

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ing cow on a lowered plane of nutrition (Shwartz et al., 2009); this is primarily characterized by low NEFA levels (Rhoads et al., 2009). Increased plasma NEFA concentrations are a classic glucose-sparing mechanism that animals on a low plane of nutrition implement to maximize milk synthesis. The fact that heat-stressed cows fail to enlist this shift in postabsorptive energetic metabolism (despite inadequate nutrient intake) may indicate that heat stress directly (not mediated by feed intake) effects energetics.

Recombinant bST (**rbST**) is a well-documented galactopoietic tool, and some of the mechanisms by which it is effective are similar to the biological strategies that poorly fed animals utilize to support copious milk synthesis. A primary component of both aforementioned situations is reduced systemic insulin action, which is a key mechanism of partitioning nutrients from extramammary tissues toward the mammary gland. Both underfed and rbST-treated lactating cows have larger NEFA responses to a lipolytic signal and slower rates of glucose disposal during a glucose tolerance test (**GTT**); these are 2 metabolic hallmarks of synchronized nutrient partitioning (Bauman, 1999). These 2 parameters indicate that both carbohydrate and lipid metabolism changes are coordinated to support the dominant physiological state of lactation. We hypothesized that rbST would override the mechanisms that prevent heat-stressed cows from mobilizing adipose lipids and thus allow the initiation of glucose-sparing events.

Over the past several decades, major advances in environmental cooling systems have helped lessen production losses during summer months (Armstrong, 1994). However, few advances have targeted strategies to improve physiological and metabolic outcomes in an effort to increase production parameters of heat-stressed dairy cattle. Study objectives were to 1) characterize the changes in various energetic and metabolic parameters that accompany heat stress exposure while eliminating the confounding effects of dissimilar nutrient intake, and 2) evaluate the effect of heat stress coupled with exogenous rbST on basal and stimulated aspects of glucose homeostasis and lipid metabolism.

MATERIALS AND METHODS

Animals and Experimental Design

Twenty-two multiparous, lactating Holstein cows (99.8 ± 20.2 DIM; 655 ± 63 kg of BW) were randomly assigned to 1 of 2 climate chambers during 3 experimental periods. The total length of the trial was 21 d, with the 3 experimental periods consisting of 1) 7 d of thermoneutral conditions and ad libitum feed intake (**P1**); 2) 7 d of either heat stress (**HS**) and ad libitum

feed intake or being pair-fed (**PF**) in thermoneutral conditions (**P2**); and 3) 7 d of either heat stress and ad libitum feed intake or being pair-fed in thermoneutral conditions, each with rbST supplementation (**P3**). Animals in the thermoneutral control conditions ($n = 12$; group 1) were pair-fed with HS animals ($n = 10$; group 2) to eliminate the confounding effects of dissimilar nutrient intake. During P2 and P3, the reduction in feed intake in heat-stressed cows was calculated daily based on the percentage decrease from the average DMI during P1; the amount offered to the PF thermoneutral cows was reduced by that amount. Cows were not receiving rbST before the start of the experiment. Recombinant bST injections (Posilac, Monsanto, St. Louis, MO) were subcutaneously administered per label instructions to all animals only on d 1 of P3. Animals were maintained in tie-stall stanchions at the University of Arizona's William J. Parker Agricultural Research Complex and all procedures were approved by the University of Arizona Institutional Animal Care and Use Committee.

All cows were individually fed a TMR 3 times daily (0500, 1200, and 1700 h) and orts were recorded daily before the a.m. feeding. During all 3 experimental periods, HS cows were allowed to eat ad libitum. However, during P2 and P3, PF cows were limit-fed as described above. The TMR was formulated to meet or exceed the predicted requirements (NRC, 2001) of energy, protein, minerals, and vitamins (Table 1). Alfalfa hay was the primary forage and steam-flaked corn was the primary concentrate. The TMR was sampled weekly and analyzed by wet chemistry methods (Chandler Analytical Laboratories, Chandler, AZ).

During P1, all cows were housed in constant thermoneutral conditions [20°C , 20% humidity, temperature-humidity index (**THI**) = 64, 12- and 12-h light and dark cycle; Figure 1). Starting on the first day of P2 and continuing until the last day of P3, HS cows experienced cyclical daily temperatures (to mimic diurnal patterns) ranging from 29.4 to 38.9°C (constant 20% humidity, **THI** = 72.4 – 82.2 , 12- and 12-h light and dark cycle; Figure 1). Cows in the PF group remained (during P2 and P3) in constant thermoneutral conditions as described for P1. The daily heat stress cyclical pattern was constant during P2 and P3 and details regarding the size and air turnover of the climate chambers have been previously reported (Rhoads et al., 2009; Shwartz et al., 2009). Respiration rate (breaths/minute, counted visually) and rectal temperatures (obtained with a GLA 525/550 Hi-Performance Digital Thermometer, San Luis Obispo, CA) were measured 4 times daily (0600, 1000, 1400, and 1800 h).

Cows were milked twice daily (0500 and 1700 h), with yields recorded at each milking. Milk samples from the

Table 1. Ingredients and chemical composition of diets¹

Item, % of DM unless noted	Value
Ingredient	
Alfalfa hay	50.3
Steam-flaked corn	25.5
Whole cottonseed	8.7
Amino plus ²	2.1
Beet pulp	4.8
Molasses	4.3
Supplement ³	2.4
Calcium salts of palm oil ⁴	1.9
Chemical analysis	
CP	16.7
NDF	26.3
ADF	18.3
NE _L , Mcal/kg of DM	1.80

¹Values represent an average of samples collected and composited throughout the trial. Diet DM averaged 50.24%.

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

³Contained 1.34% fat, 6.23% Ca, 4.49% P, 3.10% Mg, 0.58% S, 0.23% K, 16.18% Na, 3.70% Cl, 2,290.59 mg/kg of Zn, 2,037.94 mg/kg of Mn, 1,109.46 mg/kg of Fe, 629.18 mg/kg of Cu, 75.02 mg/kg of Co, 13.83 mg/kg of Se, 10.40 mg/kg of Mo, 51.65 mg/kg of I, 360.16 IU/g of vitamin A, 35.67 IU/g of vitamin D, and 1.16 IU/g of vitamin E.

⁴Maxxer; Tarome Inc., Eloy, AZ.

a.m. milking from each cow were collected at the end of each period (d 7, 14, and 21). The milk aliquots were stored at 4°C with a preservative (bronopol tablet; D&F Control System, San Ramon, CA) and analyzed by Arizona DHIA (Tempe, AZ) using approved infrared analysis, equipment, and procedures for milk components.

Body weights were obtained for all animals on d 4 of each period following the a.m. milking and were used to calculate net energy balance (EBAL) using the following equation: EBAL = net energy intake – (NE_M + NE_L).

Net energy for maintenance was calculated using the following equation: NE_M = (0.08 × BW^{0.75}; NRC, 2001). Maintenance costs were increased by 25% during the heat stress conditions as recommended (NRC, 1989). Net energy for lactation was calculated by using the following equation: NE_L = [(0.0929 × fat %) + (0.0547 × CP %) + (0.0395 × lactose %)] × milk yield (NRC, 2001).

Blood Sampling and Metabolic Challenges

Bilateral indwelling jugular catheters were inserted in all cows on d 4 of each period. Glucose tolerance tests (250 mg/kg of BW) were administered, as described previously by Mir et al. (2002), on d 5 of each period at 0800 h, immediately following the a.m. milking and feeding routine. A 50% dextrose solution (AGRIpharm Products, Grapevine, TX) was administered via the

jugular catheter and immediately chased with 12 mL of sterile saline. Blood samples were collected at –30, –20, –10, 0, 5, 7.5, 10, 20, 30, 45, 60, and 90 min relative to glucose administration. Samples were collected into disposable glass culture tubes containing 250 U of sodium heparin and were immediately placed on ice. After centrifugation, plasma was split into 2 aliquots, which were both frozen at –20°C; one aliquot was later analyzed for plasma glucose levels and the other for plasma insulin concentrations.

Daily blood samples were collected via coccygeal venipuncture (following the a.m. milking and before feeding) and the plasma was harvested and stored at –20°C until analyzed for plasma urea nitrogen (PUN), glucose, insulin, and NEFA concentrations. 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). For daily NEFA and glucose samples, the inter- and intraassay coefficients were all less than 4.8%. Inter- and intraassay coefficients for plasma glucose obtained during the GTT were 3.4 and 3.5%, respectively. The PUN concentrations were measured via the colorimetric method (Urea Nitrogen Reagent Set; TECO Diagnostics, Anaheim, CA), and the inter- and intraassay coefficients were 4.6 and 4.9%, respectively. Basal plasma insulin concentrations from d 6 and 7 of each period and from the GTT were determined using an ELISA kit (Merckodia Bovine Insulin ELISA, ALPCO Diagnostics, Salem, NH), and the inter- and intraassay coefficients were 6.4 and 6.9%, respectively, for daily samples and 5.9 and 7.1%, respectively, for samples obtained during the GTT.

Figure 1. Average diurnal patterns of temperature humidity index (THI) during thermoneutral (TN) or heat stress (HS) conditions.

Table 2. Effects of pair-feeding or heat stress and recombinant bST administration on body temperature and respiration rate in lactating Holstein cows¹

Parameter/time	Period 1		Period 2		Period 3		SEM	P-value		
	Group 1 (TN)	Group 2 (TN)	Group 1 (PF)	Group 2 (HS)	Group 1 (PF)	Group 2 (HS)		Group	Period	Group × period
Rectal, °C										
0600 h	38.38 ^{a,x}	38.28 ^{a,x}	38.19 ^{b,x}	39.47 ^{b,y}	38.44 ^{c,x}	39.88 ^{c,z}	0.09	<0.01	<0.01	<0.01
1000 h	38.27 ^{a,x}	38.37 ^{a,x}	38.38 ^{b,x}	39.56 ^{b,y}	38.54 ^{c,x}	40.02 ^{c,z}	0.10	<0.01	<0.01	<0.01
1400 h	38.56 ^{a,x}	38.58 ^{a,x}	38.35 ^{b,y}	40.43 ^{b,z}	38.38 ^{b,y}	40.55 ^{b,z}	0.08	<0.01	<0.01	<0.01
1800 h	38.46 ^{a,x}	38.51 ^{a,x}	38.52 ^{b,x}	40.55 ^{b,y}	38.55 ^{b,x}	40.80 ^{b,z}	0.08	<0.01	<0.01	<0.01
Breaths/min										
0600 h	46.5 ^{a,x}	36.6 ^{a,y}	34.7 ^{b,y}	73.0 ^{b,z}	32.0 ^{b,y}	72.3 ^{b,z}	2.2	<0.01	<0.01	<0.01
1000 h	46.5 ^{a,x}	43.5 ^{a,x}	40.3 ^{b,y}	74.6 ^{b,z}	40.0 ^{b,y}	78.2 ^{b,z}	2.0	<0.01	<0.01	<0.01
1400 h	45.4 ^{a,x}	44.3 ^{a,x}	38.0 ^{b,y}	89.2 ^{b,z}	39.1 ^{b,y}	87.3 ^{b,z}	1.8	<0.01	<0.01	<0.01
1800 h	43.8 ^{a,x}	40.9 ^{a,x}	38.6 ^{b,x}	82.2 ^{b,z}	30.1 ^{b,y}	84.5 ^{b,z}	2.2	<0.01	<0.01	<0.01

^{a-c}Values within a row with differing superscripts denote differences ($P < 0.05$) between periods.

^{x-z}Values within a row with differing superscripts denote group × period interactions ($P < 0.05$).

¹During period 1, cows in both groups were treated identically [housed in thermoneutral (TN) conditions and allowed to eat ad libitum]. During period 2, cows were either pair-fed (PF) and kept in TN conditions (group 1) or heat-stressed (HS) and allowed to eat ad libitum (group 2). During period 3, cows in both groups remained in environmental conditions as described in period 2 and were administered recombinant bST on d 1.

Calculations and Statistical Analyses

The glucose and insulin responses to the GTT were measured as area under the curve (AUC); these calculations were as described previously (Baumgard et al., 2002). Mean glucose values from the 3 samples obtained before the start of a challenge were used as the baseline metabolite concentrations, and the 0 time point sample was used for the insulin baseline value. The glucose AUC was calculated through the 60 min sample and the insulin AUC was calculated through the 30 min sample.

Effects of group, period, and their interaction were assessed as a completely randomized design using the MIXED procedure of SAS (version 9.1, SAS Institute

Inc., Cary, NC). In addition, within P2 and P3, the HS cows (group 2) were tested against the PF cows (group 1) using their respective P1 value as a covariate. For daily measurements (i.e., DMI, milk yield, metabolites), each animal's respective parameter was analyzed using repeated measures with an autoregressive covariance structure and day as the repeated effect. The model contained covariate, environment, and time (d; if a repeated measure) and an environment × time interaction (if a repeated measure). In addition, a post-hoc analysis was conducted to test differences between periods (average of d 6 and 7); the model included environment, period, and environment × period interactions. Data are reported as least squares means and considered significant if $P < 0.05$.

Table 3. Effects of pair-feeding or heat stress and recombinant bST administration on production parameters in lactating Holstein cows¹

Parameter ²	Period 1		Period 2		Period 3		SEM	P-value		
	Group 1 (TN)	Group 2 (TN)	Group 1 (PF)	Group 2 (HS)	Group 1 (PF)	Group 2 (HS)		Group	Period	Group × period
DMI, kg/d	19.29 ^a	20.39 ^a	13.80 ^b	14.26 ^b	12.99 ^b	14.06 ^b	0.50	0.03	<0.01	0.78
Milk yield, kg/d	31.36 ^{a,x}	34.74 ^{a,x}	26.57 ^{b,y}	25.18 ^{b,y}	30.84 ^{c,x}	27.62 ^{c,y}	0.91	0.58	<0.01	<0.01
Milk solids										
Fat, %	3.31	3.44	3.50	4.04	3.42	3.65	0.18	0.05	0.11	0.50
Protein, %	2.80 ^a	2.80 ^a	2.64 ^b	2.53 ^b	2.64 ^c	2.71 ^c	0.04	0.75	<0.01	0.15
Lactose, %	4.76 ^a	4.90 ^a	4.63 ^b	4.71 ^b	4.64 ^b	4.71 ^b	0.07	0.08	0.03	0.84
Milk SCC	189	29	159	30	145	30	46	<0.01	0.90	0.88

^{a-c}Values within a row with differing superscripts denote differences ($P < 0.05$) between periods.

^{x-z}Values within a row with differing superscripts denote group × period interactions ($P < 0.05$).

¹During period 1, cows in both groups were treated identically [housed in thermoneutral (TN) conditions and allowed to eat ad libitum]. During period 2, cows were either pair-fed (PF) and kept in TN conditions (group 1) or heat-stressed (HS) and allowed to eat ad libitum (group 2). During period 3, cows in both groups remained in environmental conditions as described in period 2 and were administered recombinant bST on d 1.

²For feed intake and milk yield, data presented are least squares means from d 6 and 7 of each period.

Figure 2. The effects of heat stress (HS) or pair-feeding (PF) and recombinant bST on (A) DMI and (B) milk yield in lactating cows. The mean values of the final 2 d of the thermoneutral ad libitum intake period (period 1) are represented by P1 on the x-axis. The vertical line separates period 2 from period 3. On d 1 of period 3, all cows were administered recombinant bST.

RESULTS

All cows were housed under similar thermoneutral conditions with ad libitum feed intake throughout P1. During P1, there were no treatment differences in rectal temperature or respiration rate (Table 2). At the initiation of P2, group 2 cows (HS conditions during P2) had an immediate increase [$P < 0.01$; compared with P1 and group 1 cows (PF in thermoneutral conditions during P2)] in body temperature variables (Table 2). The increase in respiration rate remained elevated in group 2 cows during P3 and did not differ from P2, but rectal temperatures in group 2 were increased ($P < 0.05$) during P3 at 0600, 1000, and 1800 h when compared with their P2 values.

Overall DMI was less ($P < 0.05$) in group 1 compared with group 2 cows, but this small difference (1.1 kg) existed in P1 and by design remained in P2 and P3 (Table 3). Heat stress conditions progressively decreased DMI for the first 5 to 6 d during P2 (a decrease of 6.15 kg;

30%) before stabilizing on d 6 and 7, and feed intake remained relatively stable and not different from P2 through P3 (Table 3; Figure 2A). By design, group 1 cows had a similar temporal feed intake pattern and the extent of decrease in DMI during P2 and P3 paralleled that of group 2 cows (Table 3; Figure 2A). There were no overall group differences ($P > 0.58$) in milk yield (Table 3), but milk production progressively decreased in both groups during P2 ($P < 0.01$) and the severity of decrease (mean of P2 d 6 and 7) was 9.56 kg (27.6%) and 4.79 kg (13.9%) for group 2 and group 1 cows, respectively. Compared with d 6 and 7 of P2, milk yield from group 1 cows increased ($P < 0.01$; 3.70 kg, 16.1% on d 6 and 7) during P3 and tended ($P < 0.07$) to increase (2.22 kg, 9.7% on d 6 and 7) in group 2 cows (Figure 2B). The response did not differ ($P > 0.2$) between groups.

Group 2 cows tended ($P > 0.05$) to have increased milk fat content, but this difference (mean of 0.3 percentage units) existed in P1 and remained in P2 and P3 (Table 3). There was no group effect on milk protein content but, compared with P1, both groups had a similar reduction ($P < 0.01$) in milk protein levels during P2 and P3 (Table 3). Milk lactose concentration tended ($P < 0.08$) to be higher in group 2 cows compared with group 1 cows, but this difference (mean of 0.1 tenths) existed during P1 and remained in P2 and P3 (Table 3). Both groups had decreased ($P < 0.05$) milk lactose levels during P2 and P3 compared with P1 (Table 3). Group 1 cows had increased ($P < 0.01$) milk SCC compared with group 2 cows, but this difference existed in P1 and remained in P2 and P3.

There was a group $\times$ period interaction on EBAL (Table 3; Figure 3), as both groups were in positive EBAL (3.95 Mcal/d) in P1 and entered negative EBAL (NEBAL) during P2 (−2.97 Mcal/d). The magnitude of NEBAL continued to progress ($P < 0.05$) during P3 for group 1 cows but remained stable (compared with P2) for group 2 cows (Table 3; Figure 3). Compared with P1, both groups of cows lost ($P < 0.01$) similar amounts (47.7 kg) of BW by d 4 of P3.

Compared with P1, both groups of cows had a reduction (9.3%; $P < 0.01$) in daily blood glucose levels during P2; this decrease remained for both groups during P3 (Table 4; Figure 4A). There was a group $\times$ period interaction ($P < 0.01$) on plasma NEFA levels, as they increased ($P < 0.01$; 102% relative to P1) in group 1 cows during P2 and further increased ($P < 0.01$; 240% relative to P1) during P3 (Table 4; Figure 4B). The NEFA levels of group 2 cows did not differ between periods (Table 4; Figure 4B). There was a group $\times$ period interaction ($P < 0.01$) on PUN levels, as both groups had increased ($P < 0.01$) concentrations during P2 (compared with P1), but group 2 cows had a larger

($P < 0.05$) response than group 1 cows (an increase of 5.84 mg/dL vs. 2.8 mg/dL; Table 4). The PUN levels remained stable in P3 for group 1 cows and, although PUN levels decreased (compared with P2) in group 2 cows, they remained increased compared with P1 ($P < 0.05$; Table 4; Figure 4C). There was a group $\times$ period interaction ($P < 0.05$) for daily plasma insulin levels, as they did not differ between periods in group 1 cows but increased (37%; $P < 0.05$) in group 2 cows during P2 and P3 compared with P1 (Table 4).

There was a period effect on the glucose AUC in response to the GTT, as P2 values were greater ($P < 0.01$) than P1 values and P3 values were larger than P2 values (Table 4). There was an overall group effect ($P < 0.01$), as group 2 cows had reduced glucose AUC compared with group 1 cows, but this difference existed in P1 (Table 4). Within P2 (and using P1 values as a covariate), group 2 cows had a decreased ($P < 0.01$) glucose AUC response to the GTT compared with group 1 cows (Figure 5), but both groups responded similarly during P3 (Figure 5). There was a group $\times$ period interaction on the insulin response to the GTT, as during P2 it decreased in group 1 cows but remained similar in group 2 cows compared with P1. During P3, the insulin response of group 1 cows increased ($P < 0.05$) and was not different than its P1 value, and the insulin AUC of group 2 cows increased ($P < 0.01$) compared with P2 (Table 5; Figure 6).

DISCUSSION

Over the past several decades, major advances in environmental cooling systems have helped to ameliorate production losses during summer months (Armstrong, 1994). However, heat stress continues to detrimentally affect the global dairy industry. Furthermore, this will

Figure 3. The effects of heat stress (HS) or pair-feeding (PF) and recombinant bST on calculated energy balance in lactating cows. The mean values of the final 2 d of the thermoneutral ad libitum intake period (period 1) are represented by P1 on the x-axis. The vertical line separates period 2 from period 3. On d 1 of period 3, all cows were administered recombinant bST.

probably become more of an issue in the future as milk yield, and thus metabolic heat production, continues to increase. This illustrates the urgent need to develop alternative strategies, potentially targeting metabolic adaptations, designed to alleviate the negative effect of heat stress on dairy cattle production. Herein, we evaluate the role of rbST, a well-established metabolic modifier, on metabolic and production parameters in a controlled heat stress environment and in cohort dairy cows maintained in thermoneutral conditions but on a similar plane of nutrition.

In the current study, all body temperature variables (at every measured time point) indicated that cows in the heat stress environment were experiencing marked hyperthermia. The HS cows had more than twice as much of a reduction in milk yield as the PF counter-

Table 4. Effects of pair-feeding or heat stress and recombinant bST administration on metabolism variables in lactating Holstein cows¹

Parameter ²	Period 1		Period 2		Period 3		SEM	P-value		
	Group 1 (TN)	Group 2 (TN)	Group 1 (PF)	Group 2 (HS)	Group 1 (PF)	Group 2 (HS)		Group	Period	Group $\times$ period
Glucose, mg/dL	73.8 ^a	74.7 ^a	66.9 ^b	67.7 ^b	70.6 ^b	68.2 ^b	1.8	0.89	<0.01	0.56
NEFA, μ Eq/L	210.0 ^{a,x}	203.5 ^{a,x}	425.1 ^{b,y}	260.7 ^{b,x}	716.8 ^{c,z}	298.3 ^{c,x}	46.7	<0.01	<0.01	<0.01
PUN ³ , mg/mL	7.76 ^{a,w}	8.25 ^{a,w}	10.56 ^{b,x}	14.09 ^{b,z}	9.58 ^{c,x}	10.84 ^{c,y}	0.40	<0.01	<0.01	<0.01
Insulin, ng/mL	0.53 ^{a,x}	0.55 ^{a,x}	0.44 ^{ab,x}	0.70 ^{ab,y}	0.53 ^{b,x}	0.78 ^{b,y}	0.05	<0.01	0.05	0.02

^{a-c}Values within a row with differing superscripts denote differences ($P < 0.05$) between periods.

^{w-z}Values within a row with differing superscripts denote group $\times$ period interactions ($P < 0.05$).

¹During period 1, cows in both groups were treated identically [housed in thermoneutral (TN) conditions and allowed to eat ad libitum]. During period 2, cows were either pair-fed (PF) and kept in TN conditions (group 1) or heat-stressed (HS) and allowed to eat ad libitum (group 2). During period 3, cows in both groups remained in environmental conditions as described in period 2 and were administered recombinant bST on d 1.

²For all parameters, data presented are least squares means from d 6 and 7 of each period.

³Plasma urea nitrogen.

parts. This agrees with our previous report (Rhoads et al., 2009), confirming that reduced nutrient intake accounts for only a portion (approximately 50%) of the decrease in milk synthesis.

Somatotropin alters tissue responsiveness to homeostatic signals and affects nutrient partitioning without apparent effects on bioenergetic parameters such as maintenance costs or the partial efficiency of milk pro-

duction (Bauman, 1999). In the current study, all PF and HS cows received rbST on d1 of P3; by the end of P3, milk yield increased by 10 and 16% for HS and PF cows, respectively. Because both PF and HS cows received rbST, our experimental design prevents us from

Figure 4. The effects of heat stress (HS) or pair-feeding (PF) and recombinant bST on (A) basal plasma glucose, (B) NEFA, and (C) plasma urea nitrogen (PUN) in lactating cows. The mean values of the final 2 d of the thermoneutral ad libitum intake period (period 1) are represented by P1 on the x-axis. The vertical line separates period 2 from period 3. On d 1 of period 3, all cows were administered recombinant bST.

Figure 5. The effects of heat stress (HS) or pair-feeding (PF) and recombinant bST on plasma glucose response to a glucose tolerance test in lactating cows. During period 1 (A), all cows experienced thermoneutral conditions with ad libitum feed intake; during period 2 (B), cows were exposed to either HS conditions and allowed ad libitum intake or thermoneutral conditions and PF; during period 3 (C), cows remained in environmental conditions as described in period 2 and were administered recombinant bST on d 1. The SEM for glucose response (area under the curve) averaged $272.67 \text{ mg/dL} \times \text{min}$ and ranged from 256.0 to 296.8 $\text{mg/dL} \times \text{min}$.

Table 5. Effects of pair-feeding or heat stress and recombinant bST administration on insulin and glucose responses to a glucose tolerance test in lactating Holstein cows¹

Parameter	Period 1		Period 2		Period 3		SEM	<i>P</i> -value		
	Group 1 (TN)	Group 2 (TN)	Group 1 (PF)	Group 2 (HS)	Group 1 (PF)	Group 2 (HS)		Group	Period	Group × period
Glucose										
Basal, mg/dL	73.0 ^a	75.3 ^a	65.1 ^b	67.3 ^b	66.3 ^b	68.2 ^b	1.7	0.13	<0.01	0.99
AUC, ² mg × dL × min	2,773 ^a	2,345 ^a	3,869 ^b	2,668 ^b	4,460 ^c	3,375 ^c	273	<0.01	<0.01	0.33
Insulin										
Basal, ng/mL	0.55 ^a	0.50 ^a	0.41 ^a	0.53 ^a	0.58 ^b	0.78 ^b	0.08	0.21	0.04	0.33
AUC, ng × mL × min	111 ^{a,x}	116 ^{a,x}	69 ^{a,y}	119 ^{a,x}	121 ^{b,x}	206 ^{b,z}	15	<0.01	<0.01	0.04

^{a-c}Values within a row with differing superscripts denote differences ($P < 0.05$) between periods.

^{x-z}Values within a row with differing superscripts denote group by period interactions ($P < 0.05$).

¹During period 1, cows in both groups were treated identically [housed in thermoneutral (TN) conditions and allowed to eat ad libitum]. During period 2, cows were either pair-fed (PF) and kept in TN conditions (group 1) or heat-stressed (HS) and allowed to eat ad libitum (group 2). During period 3, cows in both groups remained in environmental conditions as described in period 2 and were administered recombinant bST on d 1.

²Area under the curve.

eliminating acclimation as a cause for the increase in milk synthesis. However, our previous environmental experiment used a similar heat load pattern for 21 d and results indicated that milk yield gradually but continually decreased for the entire experiment (Shwartz et al., 2009). This, coupled with the fact that neither body temperature variables nor DMI adapted during P3, strongly suggests that the increase in milk yield during P3 was the result of rbST administration. Respiration rates were similar between P2 and P3 in HS cows, but rectal temperatures increased (on average of 0.37°C) at 0600, 1000, and 1800 h in rbST-treated cows. The small increase in rectal temperature during P3 (compared with P2) was probably caused by rbST because rectal temperatures did not increase with time in our other experiments (Rhoads et al., 2009; Shwartz et al., 2009). Although comparisons among studies should be made with caution, previous reports indicate that rbST-treated cows remain euthermic because of increased heat dissipation (Manalu et al., 1991). Other reports indicate that rbST increases body temperature in heat stress conditions (Elvinger et al., 1992), but this appears to be dependent on cooling status (Cole and Hansen, 1993). Regardless, the increase is probably the result of processes involved with extra milk synthesis and it would be of interest to determine whether there is a lag (similar to DMI) in rbST-stimulated sweating (Manalu et al., 1991).

Heat-stressed animals are presumed to have increased maintenance requirements because of enhanced energy expenditure for heat loss via panting and sweating and because the van't Hoff-Arrhenius equation predicts it (Fuquay, 1981; Beede and Collier, 1986). Although difficult to quantify, maintenance costs are estimated to increase by as much as 25% (NRC, 1989), and some

suggest it may be greater than 30% (Fox and Tylutki, 1998). We used a maintenance correction of 1.25 during P2 and P3 for the HS cows and, similar to previous research (Moore et al., 2005; Settivari et al., 2007; Shwartz et al., 2009), both PF and HS cows entered into a mild, calculated NEBAL during P2. During P3, the NEBAL in both groups became more severe because of an rbST-induced increase in milk yield without a corresponding increase in DMI. This was anticipated because the rbST increase in DMI lags the milk response (Bauman, 1999). The NEBAL values in P2 and P3 were supported by the fact that cows in both groups lost considerable amounts of BW (≈ 50 kg) and, by definition, animals losing BW are in NEBAL (although it is not known whether or how much reduced gut fill contributes to decreased BW).

Cows on a lowered plane of nutrition alter metabolism via homeorhetic mechanisms to support the dominant physiological state of lactation, and mobilizing adipose tissue lipids to spare glucose is one of these classic adaptations (Bauman and Currie, 1980). During P2, the PF cows had an increase in plasma NEFA levels; this was anticipated because they were on a lower plane of nutrition and in a mild state of NEBAL. In contrast, despite the reduced DMI and NEBAL, cows in heat stress conditions during P2 did not have an increase in plasma NEFA, which agrees with our recent heat stress experiments (Rhoads et al., 2009; Shwartz et al., 2009) and those of other ruminant models (Sano et al., 1983; Itoh et al., 1998). The lack of an elevated NEFA response is especially surprising because acute and chronic heat stress causes marked increases in circulating cortisol, norepinephrine, and epinephrine levels (Beede and Collier, 1986), catabolic signals that normally stimulate lipolysis and adipose triglyceride mobilization. During

Figure 6. The effects of heat stress (HS) or pair-feeding (PF) and recombinant bST on plasma insulin response to a glucose tolerance test in lactating cows. During period 1 (A), all cows experienced thermoneutral conditions with ad libitum feed intake; during period 2 (B), cows were exposed to either HS conditions and allowed ad libitum intake or thermoneutral conditions and PF; during period 3 (C), cows remained in environmental conditions as described in period 2 and were administered recombinant bST on d 1. The SEM for insulin response (area under the curve) averaged $15.2 \text{ ng/mL} \times \text{min}$ and ranged from 14.4 to $15.8 \text{ ng/mL} \times \text{min}$.

P3, the PF cows had a marked increase (69% compared with P2) in plasma NEFA consistent with rbST-induced energetic imbalance; bST is well known to increase milk yield before increasing feed intake (Bauman, 1999). In contrast, rbST administration did not increase (com-

pared with P1 and P2) plasma NEFA levels in HS cows during P3 despite increasing milk yield. This is surprising because calculated EBAL is traditionally thought to be closely associated with circulating NEFA levels (Bauman et al., 1988; Dunshea et al., 1990).

During heat stress, homeorhetic adaptations act to suppress lipid mobilization. We speculate that this is a natural and evolutionary mechanism to survive a heat load because the β -oxidation of NEFA may produce more metabolic heat than that of carbohydrates (Baumgard and Rhoads, 2007). If the opposite effect occurred during cold stress or in hypothermic animals, it would provide strong support to this hypothesis. Indeed, cold-stressed sheep (Sano et al., 2007) and cows (Tamminga and Schrama, 1998) have increased circulating NEFA and glucose levels. This agrees with rodent (Kuroshima et al., 1976; Doi et al., 1982) and human literature demonstrating an increase in fatty acid oxidation during hypothermia (Hurley and Haymes, 1982). The aforementioned metabolite changes during cold stress are probably mediated by a decrease and increase in plasma insulin and glucagon, respectively (Doi et al., 1982).

An additional metabolic hallmark of malnourished animals is either a reduction in blood insulin or a decrease in systemic insulin sensitivity (Bauman and Currie, 1980; Bauman, 1999). In this study, basal insulin values remained relatively stable in the PF cows but, despite the large decrease in nutrient intake, the HS cows actually had an increase in blood insulin levels during P2 and P3. Increased plasma insulin concentrations in the current study agree with data from another heat-stressed ruminant report (Itoh et al., 1998), a malignant hyperthermic pig model (Hall et al., 1980), and heat-stressed rodents (Torlinska et al., 1987). The increased basal insulin levels may explain the lack of an increase in basal NEFA levels in HS cows because insulin is a potent antilipolytic hormone (Vernon, 1992).

In addition to adipose tissue, skeletal muscle protein is also mobilized during periods of inadequate nutrient intake to support lactation (Bell, 1995). Compared with during P1, during P2 PF cows had a 36% increase, whereas the HS cows had a 71% increase in PUN levels. The quick increase in PUN during heat stress agrees with our recent heat stress data (Shwartz et al., 2009). Plasma urea nitrogen can originate from at least 2 sources: inefficient rumen ammonia incorporation into microbial protein, or hepatic deamination of AA mobilized from skeletal muscle. A better circulating indicator of muscle catabolism is either 3-methylhistidine or creatine, both of which are increased in heat-stressed poultry (Yunianto et al., 1997), rabbits (Marder et al., 1990), and lactating cows (Schneider et al., 1988; Kamiya et al., 2006). Why HS cows had a

more robust PUN response during P2 than PF cows is not well understood and is surprising because HS cows had much higher circulating levels of insulin, a potent antiproteolytic hormone (Allen, 1988). During P3, PUN levels remained elevated (compared with P1) but stable (compared with P2) in PF cows, and decreased (compared with P2) but remained elevated (compared with P1) in HS cows. The gradual PUN decrease in HS cows with time resembled that of our previous heat stress work (Shwartz et al., 2009), but the mechanisms responsible for the elevated PUN and its origin remain unknown.

A GTT provides an estimate of glucose disposal and systemic insulin action and can be used as a rough indicator of fuel preference. An underfed animal will typically have a slower rate of glucose disposal mediated by decreased peripheral insulin sensitivity. As expected, PF cows during P2 had an impaired ability to dispose of glucose and HS cows surprisingly had a similar (compared with P1) glucose disposal rate (Figure 5). The differences in the glucose disposal between PF and HS cows during P2 are explained by differences in the insulin response to the GTT. Compared with P1, during P2 the PF cows had a decreased insulin response, whereas the HS cows had a similar insulin response; this was similar to previous reports using insulin secretagogues in heat-stressed cows (Itoh et al., 1998). Compared with P2, the rate of glucose disposal was slower in both PF and HS cows during P3 and the insulin response to the GTT was increased in both PF and HS cows to a similar extent (Figure 6). The decreased glucose disposal and increase in insulin response to the GTT during P3 is probably caused by rbST because a reduction in systemic insulin action is a mechanism by which rbST increases nutrient delivery to the mammary gland to support enhanced milk yield (Bauman, 1999).

Clearly, the heat-stressed lactating cow experiences a variety of postabsorptive metabolic changes that are not typical of an animal on a lowered nutritional plane. With regards to lipid and carbohydrate metabolism, this is primarily characterized by the lack of adipose tissue lipid mobilization, increased basal and stimulated insulin release, and an increase in glucose disposal. The decrease in basal glucose levels agrees with multiple heat stress models (rodents, Mitev et al., 2005; poultry, Rahimi, 2005; cats, Lee and Scott, 1916; dogs, Kanter, 1954; sheep, Achmadi et al., 1993; heifers, Itoh et al., 1998; and cows, Settivari et al., 2007), and this occurs despite the increase in intestinal glucose absorptive capacity (Garriga et al., 2006), enhanced renal glucose reabsorptive capacity (Ikari et al., 2005), and elevated hepatic glucose output (Febbraio, 2001). Increased pool entry coupled with a decrease in blood glucose levels may indicate that there is an increase in the rate of

glucose leaving the circulating blood pool and suggests that glucose becomes a favored fuel for heat-stressed animals, which is supported by the increase in respiratory quotient in hyperthermic humans (Febbraio, 2001).

Cows in our current and recent heat stress studies did not mobilize adipose tissue lipids, which agreed with data on rodents (Torlinska et al., 1987), poultry (Bobek et al., 1997), pigs (Hall et al., 1980), and ruminants (Itoh et al., 1998; Ronchi et al., 1999). This lack of an NEFA response presumably indicates a reduction in fatty acid oxidation during heat stress, which has been demonstrated in heat-stressed human athletes (Febbraio, 2001). If there is a preferential need to oxidize glucose during hyperthermia, then preventing adipose tissue lipid mobilization and thus substrate competition is one logical mechanism by which this is accomplished. Elevated blood NEFA levels reduce insulin sensitivity and thus decrease muscle glucose uptake, glucose oxidation, and hepatic gluconeogenesis, which is classically referred to as the Randle effect (Randle, 1998). Collectively, minimizing adipose tissue triglyceride mobilization allows for both maximum glucose utilization and hepatic glucose output.

The increased glucose disposal and lack of an NEFA response during heat stress is probably the result of increased insulin levels, but whether this is caused by enhanced insulin secretion or diminished insulin removal is not clear. Increased pool size of any hormone or metabolite is difficult to interpret because it could be the result of increased entry (synthesis and secretion) or a decrease in pool removal. The rapid (<10 min) increase in blood insulin following the GTT suggests that the increase in basal levels is a result of enhanced pancreatic insulin release instead of decreased insulin removal. The increased pancreatic sensitivity to glucose may be one of the key metabolic adaptations animals initiate to maximize glucose utilization, and this is presumably a mechanism employed to survive a thermal load. This is supported by the fact that diabetic rats are much less likely to survive heat stress conditions compared with nondiabetic counterparts (diabetic rats have reduced capacity to utilize and oxidize glucose), and that exogenous insulin increases the likelihood of the diabetic rat surviving a thermal load (Niu et al., 2003).

CONCLUSIONS

Understanding the mechanisms by which environmentally induced hyperthermia causes decreased production and jeopardizes animal health will provide insight for developing new strategies to ameliorate the economic impact of heat stress. This study confirms our

earlier reports and clearly demonstrates that a lowered nutritional plane accounts for only approximately 50% of the reduction in milk synthesis during heat stress and that shifts in postabsorptive metabolism may be responsible for a large portion of the remainder. Heat-stressed cows have increased basal and stimulated insulin levels, resulting in decreased adipose tissue lipid mobilization and apparently increased glucose utilization by peripheral tissues. Consequently, the normal glucose-sparing mechanisms employed by thermoneutral animals to maximize milk yield during periods of nutrient insufficiency cannot be engaged by hyperthermic cows. Although this experiment characterized marked shifts in glucose and lipid metabolism, reasons why and how require further investigation.

ACKNOWLEDGMENTS

The authors express their appreciation to Rosemarie Burgos-Zimbleman, Laura Odens, Gilad Shwartz, Stephanie Celaya, Sarah Hartman, Jennifer Ernest, Shannon Carol, and Laura Hernandez for assistance at the University of Arizona's William J. Parker Agriculture Research Complex. The technical assistance of Dairy Nutrition Services (Chandler, AZ) is greatly appreciated.

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