Effects of a supplemental yeast culture on heat-stressed lactating Holstein cows¹

G. Shwartz, M. L. Rhoads, M. J. VanBaale, R. P. Rhoads, and L. H. Baumgard²

Department of Animal Sciences, The University of Arizona, Tucson 85721

ABSTRACT

Multiparous, lactating Holstein cows ($n = 23$; 120 ± 30 d in milk, 690 ± 67 kg of body weight) housed in climatic chambers were randomly assigned to 1 of 2 dietary treatments: a diet containing a novel yeast culture formulation (YC) for heat stress ($n = 12$, 10 g/d) or a control diet ($n = 11$). The trial length was 28 d and consisted of a 7-d thermal neutral period (TN; 18°C, 20% humidity) followed by 21 d of heat stress (HS; cyclical daily temperatures ranging from 29.4 to 37.8°C and 20% humidity). Cows were individually fed a total mixed ration consisting primarily of alfalfa hay and steam-flaked corn. During TN, the YC feeding had no effect on production variables or most body temperature indices. During HS, all body temperature indices increased and YC had no effect on rump surface temperature, respiration rate, or sweating rates. Cows fed YC had lower rectal temperatures at 1200 and 1800 h (40.29 vs. 40.02°C and 40.35 vs. $40.12 \pm 0.07^\circ\text{C}$, respectively) compared with control-fed cows. Cows fed both diets lost body weight (42 kg) during HS, but there were no differences between diets. Control-fed cows had increased dry matter intake (DMI) and milk yield (19.1 vs. 17.9 ± 0.5 kg/d and 32.15 vs. 29.15 ± 0.02 kg/d, respectively) compared with YC-fed cows, but intake and milk production were similar between diets when evaluated on a body weight basis. Heat stress progressively decreased DMI (29%) and milk yield, with milk production reaching a nadir (33%) in the third week. Heat stress decreased milk protein (7%) and lactose (5%) levels, but did not alter milk fat content. Heat-stressed cows were in calculated negative energy balance (-1.91 ± 0.70 Mcal/d) and this was unaffected by diet. Independent of diet, HS decreased plasma glucose (11%), but neither diet nor HS altered basal nonesterified fatty acid levels. Heat stress increased plasma urea N concentrations (11.5

vs. 14.8 ± 0.4 mg/dL). Despite YC-fed cows having slightly reduced body temperatures indices, feeding YC did not prevent the negative effects of HS.

Key words: heat stress, hyperthermia, yeast culture, metabolism

INTRODUCTION

Hyperthermia (either from pyrexia or environmentally induced) negatively affects a variety of dairy production parameters; consequently, heat stress is a costly issue to the global dairy industry (St-Pierre et al., 2003). Two of the first noticeable effects of environment-induced hyperthermia are reduced feed intake and decreased milk yield. Unabated heat stress can decrease feed intake more than 50% (Huber, 1996) and, even on well-cooled dairies, milk production can decrease by >10% (Collier et al., 1982). In addition to reduced nutrient intake, heat-stressed cows are thought to have increased maintenance costs ($\geq 30\%$; Morrison, 1983; Huber, 1996; Fox and Tylutki, 1998) as maintaining homeothermia presumably has a large energetic cost. Because of decreased energy availability and increased energy utilization, heat-stressed cows enter into a calculated negative energy balance (EBAL; Moore et al., 2005). The heat stress-induced deficit in energy and nutrient availability is thought to limit milk synthesis during a thermal load. Consequently, the energy density of the diet is often increased during warm summer months.

Methods of increasing the energy density of the diet include feeding more grains or supplemental fats. Increasing grain consumption should be conducted with caution as heat-stressed cows are already prone to rumen acidosis (Kadzere et al., 2002). Although feeding fats increased milk yield in hot conditions (Huber, 1996), there is a limit to which they can be incorporated into the diet because feeding fats, oils, and even by-pass fats can decrease fiber digestion, cause milk fat depression, and reduce DMI (Bauman et al., 2008). Alternatively, supplemental dietary performance modifiers and exogenous fibrolytic enzymes may increase digestion efficiency and provide additional energy. For example, ionophores alter microbial populations, which

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²Corresponding author: baumgard@ag.arizona.edu

results in increased propionate and decreased methane production, therefore enhancing energy conservation (Schelling, 1984). In addition, feeding yeast cultures (YC; i.e., *Saccharomyces cerevisiae*) and fibrolytic enzymes (cellulases and hemicellulases) produced by fungi and bacteria is thought to help stabilize rumen pH, enhance fiber digestion and increase postrumen nutrient flow (Erasmus et al., 1992; Beauchemin et al., 2003), all of which should benefit the heat-induced hyperthermic cow.

We hypothesized that enhancing digestion with a novel mixture of exogenous enzymes and a YC would increase energy availability and reduce the negative effect a heat-load has on milk synthesis and other production measures. Study objectives were to evaluate the effects of a supplemental dietary YC on body temperature indices, production variables, and metabolic parameters in heat-stressed lactating cows.

MATERIALS AND METHODS

Twenty-three multiparous, lactating Holstein cows (120 ± 30 DIM, 690 ± 67 of BW) were randomly assigned to 1 of 2 dietary treatments that were fed twice daily: a control TMR ($n = 11$) or the control TMR with 10 g of a novel mixture of exogenous enzymes and a YC ($n = 12$; Alltech Inc., Lexington, KY) top-dressed twice daily. The YC was a blended product containing a mixture of 2 active yeast strains with different spectra of stimulatory effects in the rumen, cobalt chloride, and a vitamin mix containing biotin and niacin. The total length of the trial was 28 d with 2 experimental periods consisting of 1) 7 d of thermal neutral (TN) conditions and 2) 21 d of heat-stress (HS) conditions. Animals were maintained in 1 of 2 environmental chambers containing 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. Each chamber housed 6 cows, contained $2,458 \text{ m}^3$ of air volume, and had an air turnover rate of $975 \text{ m}^3/\text{min}$. One half of the cows in each chamber received the control TMR and the other half received the TMR containing YC.

During period 1, all animals were housed in constant TN conditions [18°C , 20% humidity (temperature-humidity index, $\text{THI} = 64$) with a 12:12 h light:dark cycle]. Starting on the first day of period 2 (d 8) and continuing until the last day of the study (d 28), cows were subjected to cyclical daily temperatures, in an attempt to mimic daily variation, ranging from 29.4 to 37.8°C (20% humidity and a 12:12 h light: dark cycle). Between 2400 and 0700 h, the THI remained at 73; thereafter, the conditions became warmer until peaking

Table 1. Ingredients and chemical composition of diets¹

Item	% of DM
Ingredient	
Alfalfa hay	52.03
Whole cottonseed	8.43
Steam-flaked corn	29.12
Supplement ²	2.69
Maxxer ³	1.78
Amino plus ⁴	3.19
Molasses	2.78
Chemical analysis, % of DM	
CP	16.91
NDF	24.83
ADF	20.50
NE _L , Mcal/kg of DM	1.76

¹Values represent an average of samples collected and composited throughout the trial. Dry matter averaged 50% for the diet.

²The 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 IU/g of vitamin A, 30.2 IU/g of vitamin D, and 1.0 IU/g of vitamin E.

³Calcium salts of palm oil (Tarome Inc., Eloy, AZ).

⁴Soybean-based supplement; 51.7% CP (Ag Processing Corp., Hastings, NB).

at a THI of 82 (HS) between 1300 and 1500 h. After peak THI, temperatures declined until the THI again reached 73 at 2300 h. Body temperature indices were obtained 3 times daily (0600, 1200, and 1800 h) as well as respiration rates (breaths/min), surface temperature (shoulder, rump, and tail-head) recorded with an infrared temperature gun (Raynger MX model RayMX4PU Raytek, Santa Cruz, CA; accuracy $\pm 1.0^\circ\text{C}$), and body temperatures that were obtained with a rectal thermometer (GLA M700 Digital Thermometer, San Luis Obispo, CA; accuracy $\pm 0.1^\circ\text{C}$). Sweating rates were measured at the rump from a cleanly shaven area (12.9 cm^2) using a VapoMeter (Delfin Technologies Ltd., Kuopio, Finland), a device that measured the evaporation rate of water at $\pm 0.5 \text{ g/m}^2$ per hour, at 0600, 1200, and 1800 h on d 7, 14, 21, and 28.

All cows were individually fed a TMR twice daily (0500 and 1700 h), and orts were recorded before the a.m. feeding. During both experimental periods, cows were fed ad libitum. 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. Alfalfa hay was the primary forage with steam-flaked corn as the primary concentrate (Table 1). The TMR was sampled weekly and analyzed by wet chemistry methods (Dairy Nutrition Services).

Cows were milked twice daily (0500 and 1700 h) with yields recorded at each milking. Milk samples from each cow were collected on d 7, 14, 21, and 28. An aliquot

stored at 4°C with a preservative (bronopol tablet; D&F Control System, San Ramon, CA) was analyzed by Arizona DHIA (Tempe, AZ) using AOAC-approved infrared analysis equipment and procedures for milk components (fat, lactose, and protein). Body weights and BCS were obtained on all animals immediately after the morning milking and before feeding on d 1, 7, 14, 21, and 28.

Weekly blood samples were collected via coccygeal venipuncture on d 7, 14, 21, and 28 following the morning milking, and plasma was harvested and stored at -20°C until analyzed for plasma urea nitrogen (**PUN**), glucose, and NEFA concentrations. All plasma NEFA, glucose, and PUN concentrations were measured enzymatically using commercially available kits validated in our laboratory (NEFA C kit, Wako Chemicals USA, Richmond, VA; Autokit Glucose C2; Wako Chemicals USA). Plasma urea nitrogen concentrations were measured via a colorimetric method (Urea Nitrogen Reagent Set; Teco Diagnostics, Anaheim, CA). The inter- and intraassay coefficients for the NEFA, PUN, and glucose assays were 3.6 and 4.7; 3.2 and 3.5; and 4.7 and 3.1%, respectively.

Calculations

Energy balance was calculated as described (NRC, 2001) and maintenance costs were increased by 25% during the HS conditions as recommended by the NRC (1989). Daily EBAL values were subjected to a third-order polynomial regression analysis to minimize variation, and predicted daily energy values from these equations were used in the statistical analysis as described previously (Moore et al., 2004). National Research Council (2001) equations were used to calculate 3.5% FCM and ECM. Feed efficiency was calculated using the following equation: feed efficiency = 3.5% FCM/DMI. Based on treatment difference in BW (35 kg) on d 1, we evaluated milk yield and DMI as a percentage of BW.

Statistical Analysis

Daily milk yield, milk yield as a percentage of BW, DMI, DMI as a percentage of BW, EBAL, and feed efficiency were condensed into weekly values and analyzed by repeated measures using PROC MIXED (SAS Institute, 2005) with an autoregressive covariance structure and week of lactation as the repeated effect. Each animal's previous 305-d mature-equivalent milk yield was used as a covariate on all measurements. Plasma NEFA, glucose, and PUN were analyzed by repeated measures using PROC MIXED (SAS Institute, 2005) with an autoregressive covariance structure and day of

experiment as the repeated effect. The model contained chamber, day or week of experiment, treatment, and day or week of experiment by treatment interactions. Cows were the random effect, and day or week of experiment, treatment, and day or week of experiment by treatment interaction were the fixed effects. Environmental chamber was not significant and was removed from the models. Milk components were analyzed by repeated measures using PROC MIXED (SAS Institute, 2005) with an autoregressive covariance structure and day of experiment as the repeated effect. The model contained day of experiment, treatment, and day by treatment interactions. Cows were the random effect, and day of experiment, treatment, and day of experiment by treatment interaction were the fixed effects. All data were condensed into period values and the treatment, period, and treatment by period interaction were analyzed using PROC MIXED. Standard errors of the mean are reported, and differences were considered significant when $P < 0.05$ unless otherwise stated.

RESULTS

During period 1 (TN), dietary treatment had no effect on tail head temperature, rump temperature, shoulder temperature, respiration rate, and sweating rate (Table 2) at any time point. Rectal temperature tended to increase for controls compared with YC-fed cows at 1200 h (38.42 vs. 38.32°C; $P = 0.10$), but was not different at the other time points (Table 2).

Compared with period 1, during period 2 (HS), all body temperature indices were increased ($P < 0.01$) at all time points (Table 2), but dietary treatment had no effect on rump temperature, respiration rate, and sweating rate. Tail head (39.52 vs. 39.24°C; $P = 0.06$) and shoulder (39.59 vs. 39.31°C; $P = 0.08$) temperatures tended to be higher for controls at 1200 h compared with YC-fed cows, but were similar to YC-fed cows at the other 2 measurement times (Table 2). Compared with YC-fed cows, control-fed cows had increased rectal temperatures at 1200 and 1800 h (40.29 vs. 40.02°C; $P < 0.03$; 40.35 vs. 40.12°C; $P < 0.01$; Table 2). Heat stress decreased DMI (29%; $P < 0.01$; Figure 1) compared with period 1, and DMI was overall lower for YC-fed cows (19.1 vs. 17.9 ± 0.5 kg/d; $P < 0.05$; Table 3), but dietary treatment had no effect on overall DMI when evaluated as a percentage of BW ($2.76 \pm 0.08\%/d$, Table 3). Heat stress decreased milk yield (nadir = 33%; Figure 2) and all milk production variables compared with period 1. Cows fed YC had reduced milk yield compared with controls (Table 3), but there were no dietary treatment effects on milk yield as a percentage of BW, 3.5% FCM, and ECM (Table 3). Neither HS nor dietary treatment altered

Figure 1. Effects of supplemental yeast culture (YC) on DMI (kg/d) in lactating Holstein cows. Solid lines represent control-fed cows and dashed lines represent YC-fed cows. The vertical dashed line separates thermal neutral conditions (wk -1) and heat stress conditions (wk 1 to 3).

milk fat content; however, HS (but not diet) reduced milk protein (7%; $P < 0.01$) and lactose (5%; $P < 0.01$; Table 3) compared with period 1.

Body weight and BCS were greater ($P < 0.01$) in control- than YC-fed cows during the TN period (Table 3). The difference in BW and BCS between dietary treatments also existed during HS, and both BW and BCS decreased ($P < 0.01$) during HS. Diet had no effect on EBAL, but HS reduced EBAL (Figure 3) compared with period 1, and neither diet nor experimental period altered feed efficiency. Plasma glucose (Figure 4A) decreased (12%; 69.3 vs. 61.1 ± 1.2 mg/dL) with HS compared with period 1, but was similar between diets (64.5 ± 1.0 mg/dL) and neither HS nor diet affected basal

NEFA levels (145 ± 15 μ Eq/mL; Figure 4B). Thermal stress caused an immediate increase (47%; $P < 0.01$) in PUN (11.6 vs. 17.1 ± 0.4 mg/dL) compared with period 1, but during heat stress YC-fed cows tended ($P < 0.10$) to have lower PUN levels than control-fed cows (15.5 vs. 14.2 ± 0.6 mg/dL; Figure 4C).

DISCUSSION

Environmentally induced hyperthermia affects a variety of health, production, and physiological measures and is a significant animal well-being and financial burden in many, if not most, dairy-producing areas of the world. Advances in cooling system management and technology (Armstrong, 1994) have alleviated a significant portion of the negative effect that HS has on dairy cattle. Despite these advances, HS continues to reduce DMI, and this documented (Collier et al., 1982; McGuire et al., 1989) change in energy/nutrient intake is thought to be primarily responsible for the decrease in milk yield (Beede and Collier, 1986). Coupled with the reduced nutrient intake is a presumed substantial increase in maintenance costs (Beede and Collier, 1986; Fox and Tylutki, 1998). Consequently, it appears that in addition to inadequate energy intake, there is a reprioritization of postabsorptive energy utilization resulting in a rapid decline in milk synthesis. Exogenous fibrolytic enzymes may improve feed utilization and increase production, especially when energy is the first-limiting nutrient in the diet (Beauchemin et al., 2003). Therefore, we hypothesized that feeding an YC formulation containing enzymes before and during HS

Table 2. Effects of supplemental dietary yeast culture (YC) on body temperature variables in lactating Holstein cows

Parameter	Thermal neutral				Heat stress			
	0600 h	1200 h	1800 h	SEM	0600 h	1200 h	1800 h	SEM
Tail head surface, °C								
Control	31.38	31.14	32.00	0.21	35.38	39.52 ^c	38.74	0.11
YC	31.56	31.31	31.93	0.21	35.24	39.24 ^d	38.70	0.11
Rump surface, °C								
Control	31.99	31.45	32.54	0.24	35.92	39.66	39.08	0.11
YC	31.57	31.74	32.36	0.24	38.85	39.45	39.07	0.11
Shoulder surface, °C								
Control	31.31	30.83	31.94	0.26	35.42	39.59 ^c	38.88	0.11
YC	31.22	31.00	32.08	0.26	35.40	39.31 ^d	38.96	0.11
Rectal, °C								
Control	38.35	38.42 ^c	38.47	0.04	39.48 ^c	40.29 ^a	40.35 ^a	0.07
YC	38.37	38.32 ^d	38.50	0.04	39.26 ^d	40.02 ^b	40.12 ^b	0.07
Respiration, breaths/min								
Control	37.98	40.79	39.28	1.51	64.31	81.84	78.41	1.12
YC	37.24	38.39	39.66	1.51	65.05	80.31	77.33	1.12
Sweating, g/m ² per hour								
Control	29.54	29.48	29.77	4.39	45.16	72.22	62.73	4.96
YC	25.06	25.86	25.00	4.39	41.67	63.74	57.53	4.96

^{a,b}Values within columns of each variable with differing subscripts indicate $P < 0.05$.

^{c,d}Values within columns of each variable with differing subscripts indicate $0.05 < P < 0.10$.

Table 3. Effects of supplemental dietary yeast culture (YC) on production variables in lactating Holstein cows

Parameter	Thermal neutral		Heat stress			P-value		
	Control	YC	Control	YC	SEM	TRT ¹	PER	TRT × PER
DMI, kg/d	21.8	20.4	16.3	15.3	0.5	0.03	<0.01	0.76
DMI, % of BW	3.10	3.02	2.49	2.43	0.08	0.39	<0.01	0.90
Milk yield, kg/d	37.2	34.0	27.1	24.3	1.2	0.02	<0.01	0.87
Milk yield, % of BW	5.32	5.04	4.14	3.87	0.18	0.15	<0.01	0.98
3.5% FCM, kg/d	37.7	35.2	28.0	25.3	1.5	0.11	<0.01	0.96
ECM, kg/d	36.9	34.6	26.9	24.5	1.4	0.10	<0.01	0.99
Milk components								
Fat, %	3.62	3.64	3.68	3.74	0.13	0.77	0.56	0.89
Protein, %	2.81	2.89	2.59	2.69	0.05	0.10	<0.01	0.77
Lactose, %	4.78	4.67	4.56	4.54	0.05	0.28	<0.01	0.47
EBAL, ² Mcal	1.82	0.79	-2.01	-1.80	0.70	0.57	<0.01	0.39
Feed efficiency	1.74	1.76	1.71	1.70	0.07	0.94	0.43	0.65
BW, kg	693	660	653	617	12	<0.01	<0.01	0.91
BCS	2.87	2.57	2.66	2.37	0.07	<0.01	<0.01	0.96

¹TRT = YC or control diet; PER = thermal neutral or heat-stressed; TRT × PER = interaction.

²Energy balance.

would increase dietary energy extraction and prevent, or at least ameliorate, the marked decrease in milk synthesis parameters.

Cows in this experiment were severely heat-stressed as every body temperature variable measured was increased during period 2 (Table 2). We had not anticipated that feeding YC would reduce body temperature indices, but YC-fed cows had a decrease in rectal temperatures at 1200 and 1800 h during HS, despite having similar sweating rates (Table 2). The decrease in rectal temperatures during HS averaged 0.23°C and this is surprising as an increase in digestion efficiency would theoretically increase the heat increment of feeding; thus, cows fed exogenous fibrolytic enzyme could conceivably have an increase in heat production. One possible explanation is that YC-fed cows had reduced DMI and milk yield, and both variables would decrease metabolic heat production. Regardless, understanding

why YC-fed cows had a reduced body temperature and the biological and production significance (if any) of this heat savings is of interest.

Not surprisingly, the HS environment caused a marked decrease in DMI with intake nadir (~33%) occurring during the second week of thermal stress (Figure 1A). The decrease in DMI is one of the first noticeable effects of HS, and the magnitude of the decline observed in this experiment supports the literature (McGuire et al., 1989; Settivari et al., 2007). Animals decrease feed intake in an attempt to create less metabolic heat, as the heat increment of feeding, especially in ruminants, is a large portion of whole body heat production (Kadzere et al., 2002). Overall, control cows consumed more feed, but because of the nonsignificant difference in BW (35 kg), we evaluated DMI and milk yield based upon BW, and DMI as a percentage of BW did not differ between dietary treatments. Interestingly, compared with the

Figure 2. Effects of supplemental yeast culture (YC) on milk yield (kg/d) in lactating Holstein cows. Solid lines represent control-fed cows and dashed lines represent YC-fed cows. The vertical dashed line separates thermal neutral conditions (wk -1) and heat stress conditions (wk 1 to 3).

Figure 3. Effects of supplemental yeast culture (YC) on calculated energy balance in lactating Holstein cows. Solid lines represent control-fed cows and dashed lines represent YC-fed cows. The vertical dashed line separates thermal neutral conditions (wk -1) and heat stress conditions (wk 1 to 3).

second week, cows in both treatments had significantly greater feed intake during the third week of heat stress (13.9 vs. 16.0 ± 0.4 kg/d), suggesting the cows were starting to acclimate to their environment.

Similar to DMI, milk yield from YC-fed cows was lower than in controls, but this difference was eliminated when evaluated on a BW basis. Consistent with McGuire et al. (1989), milk yield progressively declined for the first 2 wk of HS and remained $\sim 33\%$ lower than TN conditions during the third week of HS (Figure 2). The decline in milk yield for the first and second weeks of HS averaged 18 and 33%, respectively, and this was almost identical to the 17 and 34% decrease in DMI during the same time (Figure 1 and Figure 2). Yet, in contrast to DMI, milk yield did not increase during the third week of heat, suggesting that although DMI may have partially recovered, postabsorptive nutrient utilization appeared to remain prioritized for extramammary purposes. The lack of parallel patterns between DMI and milk yield during the third week of HS suggests that other factors besides insufficient DMI contribute to the decrease in milk synthesis.

Neither dietary treatment nor experimental period affected milk fat content (Table 3) and this was surprising as milk from experimental HS cows (Moody et al., 1971) and commercial milk produced during the summer have reduced butter fat levels (Huber, 1996; Kadzere et al., 2002). Moreover, the effect of experimental HS on milk fat is thought dependent upon the interaction between dietary forage:concentrate ratios and temperature (Morrison, 1983). The environmentally induced reduction in milk protein content supports the literature (Kadzere et al., 2002). Treatment had no effect, but HS slightly decreased (3.6%) milk lactose levels supporting Nardone et al. (1997). It is currently unclear whether HS affects milk component synthesis directly or indirectly by influencing the delivery of component precursors.

Calculating EBAL during HS is difficult because the effects of temperature on maintenance costs have never been precisely determined (NRC, 2001). It is thought that HS increases maintenance costs (Morrison, 1983; Beede and Collier, 1986; NRC, 1989) and some have estimated the increase to be greater than 30% (Fox and Tylutki, 1998). Heat-stressed cows in the current experiment had marked reductions in DMI, but this was accompanied with similar reductions in milk yield (Figures 1 and 2). Nevertheless, using a maintenance cost correction factor of 1.25 (NRC, 1989), calculations indicated that the HS cows entered into a calculated negative EBAL (Figure 3). Dietary treatment had no effect on calculated EBAL, but the potential beneficial effects of fibrolytic enzymes are currently not incorporated into the NRC (2001) EBAL equations. The

Figure 4. Effects of supplemental yeast culture (YC) on circulating concentrations of A) glucose, B) NEFA, and C) plasma urea nitrogen (PUN) in lactating Holstein cows. Solid lines represent control-fed cows and dashed lines represent YC-fed cows. The vertical dashed line separates thermal neutral conditions (d 7) and heat stress conditions (d 14 to 28).

negative EBAL nadir (Figure 3) agrees with the DMI pattern (Figure 1) and averaged -3.52 Mcal/d during the second week of HS. The loss of BW and BCS (Table 3) supports the EBAL calculations, but how much the reduction in BW and BCS was due to a decrease in gut fill because of reduced feed intake (Figure 1) is not clear.

Dietary treatment had no effect on circulating glucose concentrations, but similar to previous ruminant experiments (Achmadi et al., 1993; Ronchi et al., 1999), HS cows had a progressive reduction ($\sim 12\%$) in blood glucose (Figure 4A). The decrease in blood glucose may explain the decreased milk lactose, as the temporal pattern of reduced glucose closely paralleled the pattern of reduced milk lactose content (data not shown). Reasons for the decrease in circulating glucose are not clear, but are probably due to the reduced DMI and decrease in hepatic supply of propionate, the primary ruminant gluconeogenic precursor. In addition, HS decreased the

total rumen VFA content (Schneider et al., 1988) and specifically propionate levels (Kelley et al., 1966). Neither diet nor environment affected circulating NEFA levels (Figure 4B) and that HS did not increase blood NEFA is surprising because calculated EBAL and NEFA levels are typically inversely correlated (Bauman et al., 1988). In other ruminant models (Sano et al., 1983; Ronchi et al., 1999), HS decreased plasma NEFA levels. It is not clear why HS cows in the current study were not mobilizing adipose tissue, even though the calculated EBAL and the loss in BW and BCS predict it, but they certainly do not appear to increase fatty acid oxidation.

Heat stress caused an immediate increase (~47%) in PUN during the first week of HS and concentrations gradually declined (although they remained significantly elevated at the third week; Figure 4C). Cows fed YC had similar PUN levels during TN conditions and the first week of HS, but had reduced concentrations during the second and third weeks of HS. The attenuation in the PUN increase during HS in YC-fed cows may be due to an improved rumen nitrogen balance (Erasmus et al., 1992). The marked decrease in milk protein yield and presumed decrease in mammary AA uptake may increase circulating AA, therefore increasing the quantity of AA needing hepatic deamination, and this might help to explain the increase in PUN. Nonetheless, the pattern of decreased milk protein yield (progressively reduced, data not shown) and that of PUN do not coincide. Alternatively, the PUN could be the result of reduced DMI or altered rumen metabolism, or inefficient ammonia incorporation into microbial protein. An additional PUN source could be muscle breakdown, but PUN is not a good marker of lean tissue catabolism. Better indicators of muscle catabolism are 3-methylhistidine or creatine (neither measured in the current study), both of which increase in HS lactating cows (Schneider et al., 1988; Kamiya et al., 2006).

CONCLUSIONS

Heat stress negatively affects almost all segments of animal agriculture and is therefore an important economic issue. We hypothesized that milk synthesis is limited by energy availability in heat-stressed cows and that exogenous fibrolytic enzymes and yeast culture supplementation would improve digestion and provide additional energy that would ameliorate the hyperthermic decline in production. Cyclic heat stress markedly increased all body temperature indices and dramatically reduced DMI and milk synthesis. Heat-stressed cows had a metabolic profile that would not have been predicted based upon their energetic state, which suggests that DMI does not completely explain

the decrease in milk yield and that postabsorptive metabolism might be involved with the heat-induced decline in production. Feeding a yeast culture did not prevent or ameliorate the negative effects of heat stress on production measures in lactating Holstein cows.

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