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The effects of heat stress on protein metabolism in lactating Holstein cows

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ABSTRACT

Heat stress (HS) decreases milk protein synthesis beyond what would be expected based on the concomitant reduction in feed intake. The aim of the present study was to evaluate the direct effects of HS on milk protein production. Four multiparous, lactating Holstein cows (101 ± 10 d in milk, 574 ± 36 kg of body weight, 38 ± 2 kg of milk/d) were individually housed in environmental chambers and randomly allocated to 1 of 2 groups in a crossover design. The study was divided into 2 periods with 2 identical experimental phases (control phase and trial phase) within each period. During phase 1 or control phase (9 d), all cows were housed in thermal neutral conditions (TN; 20°C, 55% humidity) and fed ad libitum. During phase 2 or treatment phase (9 d), group 1 was exposed to cyclical HS conditions (32 to 36°C, 40% humidity) and fed ad libitum, whereas group 2 remained in TN conditions but was pair-fed (PFTN) to their HS counterparts to eliminate the confounding effects of dissimilar feed intake. After a 30-d washout period in TN conditions, the study was repeated (period 2), inverting the environmental treatments of the groups relative to period 1: group 2 was exposed to HS and group 1 to PFTN conditions. Compared with PFTN conditions, HS decreased milk yield (17.0%), milk protein (4.1%), milk protein yield (19%), 4% fat-corrected milk (23%), and fat yield (19%). Apparent digestibility of dry matter, organic matter, neutral detergent fiber, acid detergent fiber, crude protein, and ether extract was increased (11.1–42.9%) in HS cows, as well as rumen liquor ammonia (before feeding 33.2%; after feeding 29.5%) and volatile fatty acid concentration (45.3%) before feeding. In addition, ruminal pH was reduced (9.5 and 6% before and after feeding, respectively) during HS. Heat stress decreased plasma free amino acids (AA; 17.1%) and tended to increase and increased blood, urine, and milk urea nitrogen (17.2, 243, and 24.5%, respectively). Further, HS cows had reduced plasma glucose (8%) and nonesterified fatty acid (39.8%) concentrations compared with PFTN controls. These data suggest that HS increases systemic AA utilization (e.g., decreased plasma AA and increased nitrogen excretion), a scenario that limits the AA supply to the mammary gland for milk protein synthesis. Furthermore, the increase in AA requirements during HS might represent the increased need for gluconeogenic precursors, as HS is thought to prioritize glucose utilization as a fuel at the expense of nonesterified fatty acids.
Key words: heat stress, milk protein, restricted intake, milk protein precursor, protein metabolism

INTRODUCTION

Heat stress (**HS**) induces behavioral and metabolic changes in cattle that are intended to maintain homeothermy (West, 1994), often at the expense of decreased productivity and profitability. For instance, HS animals reduce DMI, activity, and metabolic rate in an attempt to decrease metabolic heat production (NRC, 2001). In dairy cattle, HS decreases milk yield (Bandaranayaka and Holmes, 1976; Rhoads et al., 2009; Wheelock et al., 2010; Cowley et al., 2015), which has been traditionally attributed to the heat-induced reduction in DMI (Fuquay, 1981; Beede and Collier, 1986; West, 2003). However, the utilization of pair-fed thermal neutral (**PFTN**) controls demonstrated that reduced DMI only partially (about 50%) explains the decrease in productivity, suggesting that hyperthermia itself directly affects milk production (Bandaranayaka and Holmes, 1976; Wheelock et al., 2010; Cowley et al., 2015).

Milk composition is also discordantly altered during hyperthermia, which indicates that HS regulates component synthesis in addition to its overall effect on milk yield (Bernabucci et al., 2015; Cowley et al., 2015). For instance, HS decreases milk protein content and yield, but the involved mechanisms remain largely unknown. Rhoads et al. (2009) suggested that modest changes in the somatotropic axis may explain a small portion

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Table 1. Experimental design

Treatment	Period 1 (18 d)		Wash-out period (30 d)	Period 2 (18 d)	
	Control (phase 1)	Trial (phase 2)		Control (phase 1)	Trial (phase 2)
Heat stress	—	Group 1	—	—	Group 2
Thermal neutral and pair-fed	—	Group 2	—	—	Group 1
Thermal neutral and ad libitum	Group 1 Group 2	—	Group 1 Group 2	Group 1 Group 2	—

of the reduction in milk protein yield during HS. Further, Cowley et al. (2015) demonstrated that the heat-induced reduction in milk protein of heat-stressed cows is the result of the specific downregulation of mammary protein synthetic activity and not an artifact of the overall milk yield reduction. Understanding the biology behind the adaptation to hyperthermia is critical to develop management, nutritional, and pharmacological strategies to mitigate its deleterious effects on productivity. The current study objective was to elucidate the mechanisms by which HS directly reduces milk protein concentration. For this purpose, we investigated HS effects on production, digestibility, rumen fermentation, and blood/urine nitrogen parameters relative to PFTN controls.

MATERIALS AND METHODS

Animals and Experimental Design

Animals were handled and cared for following the guidelines of the Institute of Animal Science, Chinese Academy of Agricultural Sciences. Four multiparous, lactating Holstein cows (101 ± 10 DIM, 574 ± 36 kg of BW, 38 ± 2 kg of milk/d, second parity, 1–2 mo pregnant) were individually housed in environmental chambers and randomly allocated to 1 of 2 groups in a 2 × 2 crossover design (Table 1). The study was divided into 2 periods (period 1 and period 2) with 2 identical experimental phases (control phase and trial phase) within each period. During phase 1 or control phase (9 d), all cows were in thermal neutral conditions [**TN**; 20°C, 55% humidity; temperature-humidity index (**THI**) = 65.5] and fed ad libitum. During phase 2 or treatment phase (9 d), group 1 (n = 2) was exposed to cyclical HS conditions (0600–1800 h at 36°C, 1800–0600 h at 32°C, 40% humidity; THI = 84.5) and fed ad libitum, whereas group 2 remained in TN conditions but was pair-fed (**PFTN**) to their HS counterparts. To calculate the amount of feed offered to the PFTN cows based on the intake of HS cows, the trial (sampling and feed restriction) started and ran 1 d behind HS cows for the PFTN cows as previously described (Wheelock et al., 2010). For the pair-feeding calculations, the ad libitum control phase daily feed intake was averaged for each cow and used as a baseline. For each HS cow, the decrease in feed intake during the treatment phase was calculated as the percentage of feed intake reduction relative to the control phase for each day of HS exposure. This percentage of feed intake reduction was averaged for all the HS cows per day of exposure and applied individually to the baseline of each PFTN cow. After a 30-d washout period in TN conditions, the study was repeated (period 2), inverting the environmental treatments of the groups relative to the treatment phase in period 1. Cows were kept in a 12 h light:12 h dark cycle and allowed to drink water ad libitum. Cows were fed a TMR formulated to meet or exceed the predicted requirements (NRC, 2001) of energy, protein, minerals, and vitamins (Table 2). All cows were individually fed twice daily (0500 and 1700 h) and orts were recorded daily before the morning feeding. During the treatment phase, calculated feed for the PFTN animals was divided in 2 and offered following the same schedule. Throughout the experiment, cows were milked twice daily (0500 and 1700 h) and milk yields were recorded at each milking.

Sampling

Feed samples from the morning TMR and the orts were collected on d 2, 4, 6, and 8 of each phase, composited, and frozen at −20°C for further analysis. Body weight was measured 1 d before and at the end of each period, using a weighbridge (Mettler Toledo Ltd., Zurich, Switzerland).

On d 5 and 6 of each phase, total urine and feces were collected separately. Urine was obtained using a device that covered the vulva (but not the anus) connected through a tube to a collection container. To increase the accuracy of urine nitrogen content, we measured the collected volume every 4 h and stored 10% of the urine (the remainder was discarded) at 4°C. At the end of the 2-d sampling period, we mixed all the urine per cow and sampled 50 mL that was acidified with 0.5 mL of 6 *M* HCl. Feces excreted during the 24 h were weighed in the morning, homogenized, sampled (~400 × *g*), and stored at 4°C until the end of the feces sam-