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Evaluation of dietary betaine in lactating Holstein cows subjected to heat stress

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ABSTRACT

Betaine (BET), a natural, organic osmolyte, improves cellular efficiency by acting as a chaperone, refolding denatured proteins. To test if dietary BET reduced the effect of heat stress (HS) in lactating dairy cows, multiparous, lactating Holstein cows (n = 24) were blocked by days in milk (101.4 ± 8.6 d) and randomly assigned to 1 of 3 daily intakes of dietary BET: the control (CON) group received no BET, mid intake (MID) received 57 mg of BET/kg of body weight, and high dose (HI) received 114 mg of BET/kg of body weight. Cows were fed twice daily and BET was top-dressed at each feeding. Cows were milked 2 times/d and milk samples were taken daily for analysis. Milk components, yield, feed intake, and water intake records were taken daily. Rectal temperature and respiration rate were taken 3 times/d at 0600, 1400, and 1800 h. Cows were housed in environmentally controlled rooms and were allowed acclimation for 7 d at thermoneutral (TN) conditions with a mean temperature-humidity index of 56.6. Cows were then exposed to 7 d of TN followed by 7 d of HS represented by a temperature-humidity index of 71.5 for 14 d. This was followed by a recovery period of 3 d at TN. Dietary BET increased milk yield during the TN period. No differences were found between BET and CON in total milk production or milk composition during HS. The increase in water intake during HS was not as great for cows fed BET compared with controls. The cows on CON diets had higher p.m. respiration rate than both MID and HI BET during HS, but lower rectal temperature compared with BET. No difference was found in serum glucose during TN, but cows given HI had elevated glucose levels during HS compared with CON. No differences were found in serum insulin levels between CON and BET but an intake by environment interaction was present with insulin increasing in HI-treated lactating dairy cows during HS. The heat shock response [heat shock protein (HSP) 27 and HSP70] was upregulated in bovine mammary epithelial cells in vitro. Blood leukocyte HSP27 was downregulated at the HI dose under TN conditions and HSP70 was upregulated at the HI dose and this effect was increased by HS. No effect was seen with the MID dose with HSP27 or HSP70. The lack of effect of BET at MID may be associated with uptake across the gut. We conclude that BET increased milk production under TN conditions and was associated with reduced feed and water intake and slightly increased body temperatures during HS of cows fed BET. The effect of BET on milk production was lost during HS with HI BET, whereas serum glucose levels increased during HS.
Key words: dairy cow, betaine, heat stress, blood glucose

INTRODUCTION

Betaine (**BET**; trimethylglycine) is a zwitterion that has many activities that may reduce the effect of heat stress (**HS**) in lactating dairy cows and improve general production. These include the fact that betaine (**BET**) is an organic osmolyte (Hammer and Baltz, 2002), is a molecular chaperone (Sharma et al., 2009), has been shown to decrease susceptibility of microbial populations to stress (Lai and Lai, 2011), acts as an antimicrobial to some bacteria such as *Salmonella typhimurium* (Lindstedt et al., 1990), can be used as a nutrient (Craig, 2004), and has been demonstrated to increase milk production when fed (Wang et al., 2010). Betaine is transported across cell membranes utilizing a Na (+)-coupled betaine-specific transporter of the betaine-choline-carnitine transporter family involved in the response to hyperosmotic stress (Perez et al.,

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2011). This osmoregulation is also seen in microbial populations and has been shown to promote favorable bacterial growth under osmotic stress conditions (Diamant et al., 2001; Wdowiak-Wrobel et al., 2013) including fluctuations in pH (Laloknam et al., 2006). Betaine has a net neutral charge, but has a region of positive and negative charges. This allows BET to hold water molecules (intracellular) against a concentration gradient, yet the affinity is not so much that the water is unavailable to the cell.

Osmolytes like BET can prevent heat-induced damage by stabilizing cellular proteins, refolding unfolded peptides and allowing the disaggregation and refolding of heat-damaged proteins (Diamant et al., 2003). Glycine BET has been shown to increase the metabolic efficiency of glucose in *Escherichia coli* cells stressed with salt (Metris et al., 2014). Another advantage that organic osmolytes have over salts is that they do not interfere with native enzymatic activity within cells (Nakanishi et al., 1990).

Betaine has 2 potential sites of action in lactating ruminants: mammalian cells and the microbes in the gastrointestinal tract. Under different types of stress, both microbial and animal cells increase uptake of exogenous BET (Nakanishi et al., 1990). The benefits of supplementing BET in lactating dairy cow diets have been demonstrated under thermoneutral (**TN**) conditions. These include increased milk production (Wang et al., 2010; Peterson et al., 2012), increased VFA production, and higher FCM yield (Wang et al., 2010). Rumen microbial utilization and degradation of BET would limit availability to rumen epithelial or other nondigestive tract cell types. However, Nakai et al. (2013) indicated that fed BET is found in the duodenal digesta, indicating that some BET escapes the rumen.

We hypothesize that dietary BET will reduce the effect of HS on rectal temperature and respiration rate and improve cellular thermotolerance by increasing heat shock protein (**HSP**) production in vivo in mammary epithelial cells and white blood cells. Furthermore, this improvement in thermotolerance would result in greater production of lactating dairy cows. The objective of this study was to evaluate the role of dietary BET in improving thermotolerance in lactating heat-stressed dairy cows.

MATERIALS AND METHODS

Cell Culture

Primary bovine mammary epithelial cells (**BMEC**) were harvested, isolated, and prepared according to Stiening et al. (2008). The cells were thawed from liquid nitrogen storage, suspended in Dulbecco's modified Eagle medium/F-12 (Gibco, Life Technologies, Grand Island, NY), mixed in neutralized collagen, and cultured (Stiening et al., 2008) in 24-well plates (Falcon, BD Biosciences, San Jose, CA). The collagen was added in 2 steps, as the base layer was allowed to gel for 5 min and the second layer containing the cells was seeded into each well in a final volume of 500 μL. The BMEC were grown in collagen that was extracted from rat-tails and allowed to grow at 37°C for 7 d (McGrath, 1987; Stiening et al., 2008) with media changed every 48 h.

The serum-free Dulbecco's modified Eagle medium/F-12 medium contained 0.1% BSA, antibiotic-antimycotic (100 U/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B; 15240, Invitrogen Corp., Carlsbad, CA). The added growth factors included IGF-1 (recombinant human, 100 ng/mL, NIDDK, Torrance, CA), epidermal growth factor, (EGF, recombinant human, 25 ng/mL, Invitrogen Corp.), and 500 ng of progesterone (Sigma-Aldrich, St. Louis, MO; Hernandez et al., 2011) per 100 mL of medium.

On d 8, cells were treated with 0 or 25 m*M* BET within the same culture plate, with one set of plates being subjected to HS (42°C) and the other set remaining at TN (37°C) for 24 h. Cells were continuously maintained at these temperatures for 24 h and then harvested. There were 6 wells per BET treatment per environment. Two wells of each subset (treatment and environment) were combined and placed in TRIzol reagent (Life Technologies, Carlsbad, CA) so that there were triplicate biological samples. Ribonucleic acid was isolated from triplicate samples, treated with DNase (DNase I, Invitrogen Corp.), cDNA was synthesized (iScript cDNA Synthesis kit, Bio-Rad, Hercules, CA), and quantitative real-time PCR using the Bio Rad iQ5 optical system (Bio-Rad) was conducted following the procedures described by Hernandez et al. (2011). The expression of HSP70 and HSP27 was quantified using the calibrator gene 40S ribosomal protein S9 (RPS9) for calculating gene expression data based on the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). A one-way ANOVA was conducted on ΔCt (cycle threshold) value with the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Data are presented as fold change.

Lactation Study

This study was conducted on a protocol approved by the Institutional Animal Care and Use Committee of the University of Arizona. Cows for this study were obtained from Caballero Dairy, Eloy, Arizona. Twenty-four multiparous Holstein cows were blocked by DIM (101.4 ± 8.6 d) and randomly assigned to treatment