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Lowering rumen-degradable and rumen-undegradable protein improved amino acid metabolism and energy utilization in lactating dairy cows exposed to heat stress

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ABSTRACT

The objective of this study was to evaluate the effects of reducing dietary rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) on protein and energy metabolism in heat-stressed dairy cows. Eighteen primiparous and 30 multiparous mid-lactation Holstein cows were used in a completely randomized design arranged in a 2 × 2 factorial (n = 12/treatment). Cows were randomly assigned to 1 of 4 dietary treatments that included 2 levels of RDP (10 and 8%; D) and 2 levels of RUP (8 and 6%; U) of dry matter for 21 d as (1) 10D:8U, (2) 8D:8U, (3) 10D:6U, and (4) 8D:6U. Diets were isoenergetic and contained 50% forage and 50% concentrate (dry matter basis). Cows were housed in a freestall barn. Three weeks before start of treatments, all animals were fed the 10D:8U diet and received supplemental cooling to prevent heat stress. During the treatment period, cows experienced a daily increment in temperature-humidity index from 74 to 82 for 1000 to 2000 h. Blood samples were collected on d −1 and 21 of the treatment period to determine plasma concentrations of AA, glucose, insulin, fatty acids, and β-hydroxybutyrate. For primiparous cows, reducing from 10 to 8% RDP decreased insulin concentrations. For multiparous cows, we found significant RDP by RUP interactions for insulin, β-hydroxybutyrate, fatty acids, total essential AA, and 3-methylhistidine concentrations. Reducing from 10 to 8% RDP decreased insulin concentrations at 6% RUP, but concentrations did not change when reducing RDP at 8% RUP. Reducing from 10 to 8% RDP decreased β-hydroxybutyrate concentrations at 8% RUP, but concentrations did not change when reducing RDP at 6% RUP. Reducing from 10 to 8% RDP increased nonesterified fatty acid and total essential AA concentrations at 8% RUP, but concentrations did not change when reducing RDP at 6% RUP. Reducing from 8 to 6% RUP decreased 3-methylhistidine concentration at 8% RDP, but not at 10% RDP. Reducing from 8 to 6% RUP increased milk protein yield efficiency in primiparous and multiparous cows. These results indicate that reducing RDP and RUP lowers circulating insulin, which was associated with mobilization and utilization of fatty acids. Reduced RDP and RUP increases the use of AA to maintain milk protein synthesis and limit AA catabolism in cows exposed to warm climates.
Key words: amino acid, dairy cow, heat stress

INTRODUCTION

Dairy cows in negative energy balance rely on energy reserves to maintain homeostasis because the demand for energy exceeds that of energy intake; thus, fatty acids are mobilized from stored triglycerides in adipose tissue (Bell, 1995). During negative energy balance, low circulating insulin levels facilitate lipolysis and utilization of fatty acids in peripheral tissues (Bell, 1995; Randle, 1998). Lactating cows exposed to short-term periods of high environmental temperatures and humidity enter into negative energy balance. Although animals exposed to heat stress reduce feed intake, circulating insulin levels increase as part of an adaptive mechanism, altering metabolism of carbohydrates, lipids, and proteins (Rhoads et al., 2009; Wheelock et al., 2010). The cause for the rise of circulating insulin is not clear during heat stress, but elevated insulin, a known promoter of glucose uptake in skeletal muscle and adipose tissues, limits fuel availability for milk synthesis during heat stress. For example, the insulin promotes glucose disposal in peripheral tissues on heat-stressed animals (Rhoads et al., 2009; Sanz Fernandez et al., 2015). Therefore, metabolic and physiological adaptations during heat stress promote the use of nutrients in peripheral tissues and may reduce nutrient availability for milk synthesis (Rhoads et al., 2009; Wheelock et al., 2010).

As circulating glucose levels in ruminants are relatively low, particularly in early-lactation cows, insulin secretion is primarily regulated by rumen production

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and absorption of propionate (Manns and Boda, 1967). In addition to propionate, levels of MP supply control both secretion and plasma concentrations of insulin in growing heifers (Wiley et al., 1991) and lactating cows (Rius et al., 2010). For example, heifers supplemented with 250 g/d of additional RUP increased secretion of insulin compared with a control diet (Wiley et al., 1991). Moreover, late-lactation primiparous cows supplemented with rumen-protected AA (i.e., 300 g/d of formaldehyde-treated casein) showed a 40% increase in plasma insulin concentrations compared with non-supplemented cows, and greater insulin concentrations were negatively correlated with milk and milk component yields (Hunter and Magner, 1988). Collectively, the increased circulating concentrations of insulin in these studies were likely caused by an increase in AA concentrations because insulin secretion responds to changes in plasma AA profile and concentrations (Kuhara et al., 1991). Therefore, with regard to the present study, we hypothesized that reduction of RDP and RUP would reduce plasma insulin concentrations and maintain nutrient availability to sustain synthesis of milk and milk components in heat-stressed lactating dairy cows. The objective of our study was to assess the effects of RDP and RUP on metabolic parameters that influence protein and energy use in lactating dairy cows.

MATERIALS AND METHODS

Animals, Housing, and Treatments

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Tennessee. Animals and experimental design of the study were previously described by Kaufman et al. (2017). Briefly, 18 primiparous (140 ± 49 DIM) and 30 multiparous (126 ± 47 DIM) Holstein cows were housed in a freestall barn at the East Tennessee AgResearch and Education Center, Little River Animal and Environmental Unit (Walland, TN). In a completely randomized design, cows were stratified into 4 treatment groups based on DIM, parity (~5 primiparous and 8 multiparous cows/treatment group), milk production, and BCS and randomly assigned to receive 1 of the 4 treatment diets arranged in a 2 × 2 factorial. Dietary treatments were formulated with 2 levels of RDP (10 and 8%; D) and 2 levels of RUP (8 and 6%; U) on a DM basis as (1) **10D:8U**, (2) **8D:8U**, (3) **10D:6U**, and (4) **8D:6U**. Diets were formulated to contain 50% forage and 50% concentrate on a DM basis, with the protein proportions manipulated by varying quantities of soybean meal, protected soybean meal (SoyPLUS; West Central Cooperative, Ralston, IA), fish meal, blood meal, and urea according to NRC (2001). Dietary composition was previously reported (Kaufman et al., 2017). Diets were mixed as a TMR and fed to each individual cow at 0900 h using an electronic feeding system (American Calan Inc., Northwood, NH). Feed was offered to attain 5 to 10% refusals each day. Cows were fed the 10D:8U diet from d 1 through 21 of the pretreatment period, followed by the respective experimental diets for an additional 21 d (i.e., treatment period). The NRC (2001)-predicted AA profiles as a proportion of MP from each dietary treatment are displayed in Table 1. Cows were milked twice daily at 0900 and 1900 h and milk production was automatically recorded at each milking. Milk sampling and analysis were previously reported (Kaufman et al., 2017).

Environmental Management

During the pretreatment period, cows received supplemental cooling by using fans starting at >20°C of ambient temperature. Throughout the treatment period, all cows experienced 10 h of unabated daily summer temperatures from 1000 and 2000 h. At 2000 to 1000 h, temperature-humidity index (**THI**) ranged from 69 to 76; thereafter, the environment was at a THI range of 74 to 82. Core body temperature and respiration rates were recorded to monitor thermal load during the treatment period and reported elsewhere, demonstrating cows were successfully exposed to heat stress (Kaufman et al., 2017).

Blood Collection and Analyses

Blood samples were collected from each cow by coccygeal venipuncture, after the morning milking, on d −1 and 21 of the treatment period in blood tubes containing 140 IU of sodium heparin (Becton Dickinson and Co., Franklin Lakes, NJ). Plasma was harvested from each sample by centrifugation at 1,500 × *g* for 20 min at 4°C, and plasma aliquots were stored at −80°C while awaiting subsequent analyses for metabolites (AA, BHB, glucose, and fatty acids) and insulin (Garverick et al., 2013; McCarthy et al., 2015). Concentrations of plasma BHB (Sigma-Aldrich, St. Louis, MO), glucose (Sigma-Aldrich), and fatty acids (Wako Diagnostics, Mountain View, CA) were determined using commercially available kits through microplate spectrophotometry (Synergy H1 Multi-Mode Reader; BioTek, Winooski, VT). Concentrations of plasma insulin were determined by double-antibody RIA with 90% cross-reactivity to bovine insulin (EMD Millipore