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Effects of reduced dietary protein and supplemental rumen-protected essential amino acids on the nitrogen efficiency of dairy cows

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ABSTRACT

When fed to meet the metabolizable protein requirements of the National Research Council, dairy cows consume an excess of N, resulting in approximately 75% of dietary N being lost to the environment as urine and feces. Reductions in environmental N release could be attained through an improvement in N efficiency. The objective of this study was to determine if the predicted reduction in milk yield associated with feeding a low-protein diet to lactating dairy cows could be avoided by dietary supplementation with 1 or more ruminally protected (RP) AA. Fourteen multiparous and 10 primiparous Holstein cows, and 24 multiparous Holstein × Jersey crossbred cows were used in a Youden square design consisting of 8 treatments and 3 periods. The 8 dietary treatments were (1) a standard diet containing 17% crude protein [CP; positive control (PC)], (2) a 15% CP diet [negative control (NC)], (3) NC plus RP Met (+M), (4) NC plus RP Lys (+K), (5) NC plus RP Leu (+L), (6) NC plus RP Met and Lys (+MK), (7) NC plus RP Met and Leu (+ML), and (8) NC plus RP Met, Lys, and Leu (+MKL). Dry matter intake was not affected by treatment. Crude protein intake was lower for NC and RP AA treatments compared with the PC treatment. No detrimental effect was detected of the low-CP diet alone or in combination with AA supplementation on milk and fat yield. However, milk protein yield decreased for NC and +MKL diets, and lactose yield decreased for the +MKL compared with the PC diet. Milk urea N concentrations were lower for all diets, suggesting that greater N efficiency was achieved by feeding the low-protein diet. Minimal effects of treatments on arterial plasma essential AA concentrations were detected, with only Ile and Val being significantly lower in the NC than in the PC diet. Phosphorylation ratios of signaling proteins known to regulate mRNA translation were not affected by treatments. This study highlights the limitations of requirement models aggregated at the protein level and the use of fixed postabsorptive efficiency to calculate milk protein requirements. Milk protein synthesis regulation by signaling pathways in vivo is still poorly understood.
Key words: nitrogen efficiency, rumen-protected essential amino acid, milk protein

INTRODUCTION

Dairy cows have requirements for specific EAA during lactation. However, current N requirements (NRC, 2001) are expressed in terms of MP rather than individual EAA. By feeding to meet MP requirements, animals are likely overfed several EAA to ensure meeting key EAA requirements across a range of diets, which leads to poor N efficiency. Approximately 25% of the N consumed by dairy cows is converted into milk protein, whereas the remaining N is excreted in urine and feces (Tamminga, 1992; Chase, 1994; Hristov et al., 2004). Feeding animals low-protein diets is one way to improve N efficiency (Kalscheur et al., 2006), but the practice is typically associated with decreased production, which is economically disadvantageous to producers. The production losses observed when MP supply is reduced are likely associated with a deficiency of 1 or several EAA, and supplementation of those deficient EAA may maintain production. Improving N efficiency by feeding reduced-protein diets and supplementation with only a few EAA should reduce N excretion to the environment and, depending on the cost of the reformulated diet with supplemental EAA, may be more economical.

Defining EAA requirements for dairy cows is especially difficult compared with monogastrics because of the use of a variety of feed products and the alteration of nutrients in the rumen by microorganisms (Lapierre et al., 2006). A better understanding of EAA requirements is needed to ensure successful application of low-protein diets. Lysine and Met have been generally accepted to be the main EAA limiting milk protein synthesis from corn-based diets (Clark, 1975; Schwab et al., 1976; NRC,

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2001). These observations have led to extensive research evaluating the supplementation with rumen-protected (**RP**) Lys and Met products. Observed responses indicate that cows fed both RP Met and Lys may increase milk protein yield depending on dietary CP supply (Robinson et al., 1998; Patton, 2010).

In addition to Met and Lys, dairy cows may respond to Leu supplementation. Leucine has been shown to increase protein synthesis rates in skeletal muscle of growing pigs (Escobar et al., 2006) and milk protein synthesis rates in mammary tissue slices (Appuhamy et al., 2012). Amino acids, in particular Leu, increase phosphorylation of mammalian target of rapamycin (**mTOR**), which controls protein synthesis rates (Wang and Proud, 2006; Appuhamy et al., 2012). Therefore, including RP Leu in dairy cow rations may result in increased milk protein synthesis through the activation of the mTOR pathway. Duodenal infusions of 40 g of Leu/d to corn-based diets improved both milk protein content and yield on diets limiting in Lys or Met (Rulquin and Pisulewski, 2006). However, jugular infusion of Ile, Leu, and Val to cows fed an MP-deficient diet (16% CP), but sufficient in Lys and Met, did not further increase milk protein yield (Appuhamy et al., 2011b).

The objectives of this study were to (1) determine the degree of reduction in milk production when MP supply is reduced by 15%; (2) determine whether the reduction in milk production associated with feeding an MP-deficient diet could be alleviated by supplementation of RP Met, Lys, Leu, or combinations of the RP AA; (3) determine the effects of feeding RP Lys and Met on cell signaling proteins associated with the mTOR pathway; and (4) to assess the effect of MP-deficient diets supplemented with RP AA on N efficiency of dairy cows.

MATERIALS AND METHODS

Animals

All experimental procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee (Blacksburg). Fourteen multiparous and 10 primiparous Holstein cows, and 24 multiparous Holstein × Jersey crossbred cows (189 ± 79 DIM; 566 ± 50 kg BW) were used in a Youden square design consisting of 8 treatments and 3 periods. Cows were randomly assigned to treatments. Experimental periods lasted 15 d and consisted of 10 d of diet adaptation and 5 d of sample collection. Cows were housed in a freestall barn with Calan Broadbent individual animal feeders (American Calan Inc., Northwood, NH) and free access to water. Four cows were removed from the study for reasons unassociated with the treatments.

Treatments

The 8 dietary treatments were (1) a standard diet containing 17% CP [positive control (**PC**)] formulated to meet requirements of a lactating cow producing 35 kg/d,(2) a 15% CP diet [negative control (**NC**)], (3) NC diet plus RP Met (**+M**), (4) NC diet plus RP Lys (**+K**), (5) NC diet plus RP Leu (**+L**), (6) NC diet plus RP Met and Lys (**+MK**), (7) NC diet plus RP Met and Leu (**+ML**), and (8) NC diet plus RP Met, Lys, and Leu (**+MKL**). Final diets contained 46% forage and 54% concentrate, and 75% of the diet ingredients were corn based (Tables 1 and 2). Three concentrate mixes were used in the experiment. A moderate-fat grain with high CP was used for the PC diet, a moderate-fat grain with low CP was used for the NC diet, and a low-fat grain with low CP was used in conjunction with the moderate-fat, low-CP mix to formulate the diets supplemented with AA. The moderate-fat low-CP and the low-fat low-CP concentrates were mixed in varying proportions to formulate the +AA diets so that dietary fat was similar across treatments. Ingredient compositions of the experimental diets are presented in Table 3. Diets were mixed as a TMR, provided in quantities to maintain between 5 and 10% refusals, and delivered to the Calan gates once daily by 1400 h. Rumen-protected AA were provided at 0.7 g of RP DL-Met/kg of DM (70.6% Met in DM; Balchem Corp., New Hampton, NY), 2.1 g of RP L-Lys/kg of DM (32.1% Lys in DM; AminoShure-L; Balchem Corp.), and 7.8 g of RP L-Leu/kg of DM (53.9% Leu in DM; source: Ajinomoto Co. Inc., Tokyo, Japan, protected by Balchem Corp.). These amounts were estimated to be adequate to achieve absorbed individual AA supplies that were equivalent to the PC diet.

Sample Collection and Analysis

Feed offered and orts were recorded daily to determine DMI. Dry matter content was determined weekly on the major components of the TMR (grain mixes, alfalfa haylage, and corn silage) and used to adjust the ration to maintain the targeted DM inclusion rates. Samples of the TMR and forage mix were collected on d 11, 13, and 15 of each period. Feed samples were stored at −20°C for later analysis. Individual feed samples were thawed and dried at 55°C in a forced-air oven for 48 h to determine DM concentrations. Dried samples were ground in a Wiley mill through a 1-mm screen (Arthur H. Thomas Co., Philadelphia, PA), and subsequently composited by diet and period. Composited samples were submitted to Dairyland Laboratories Inc. (Arcadia, WI) for nutrient analyses.