J. Dairy Sci. 98:1–10
http://dx.doi.org/10.3168/jds.2015-9819

Branched-chain amino acid and lysine deficiencies exert different effects on mammary translational regulation

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ABSTRACT

Deficiencies and imbalances of specific group II essential amino acids (EAA) were created in lactating cows by an infusion subtraction protocol to explore effects on milk production and abundance and phosphorylation state of regulators of mRNA translation in the mammary glands. Five lactating cows on a diet of 11.2% crude protein were infused abomasally for 5 d with saline, 563 g/d of a complete EAA mix, or EAA mixes without the branched-chain amino acids (BCAA), Leu, or Lys in a 5 × 5 Latin square design. Milk protein yield was stimulated by EAA infusion and returned to saline levels upon subtraction of BCAA, Leu, or Lys. Mammary abundance of phosphorylated S6K1 was measured as an indicator of mammalian target of rapamycin complex 1 (mTORC1) activity and was found not to be affected by the complete EAA mix but was increased by the mixture lacking Lys. Total S6K1 abundances in mammary tissue were elevated by complete and BCAA-lacking infusions. All of the EAA treatments except the one lacking BCAA upregulated mammary eIF2Bε and eIF2α abundances, which is stimulatory to global mRNA translation. Phosphorylation state of eIF2Bε tended to decrease when complete or Lys-lacking EAA mixtures were infused. Phosphorylation state of eIF2α was not affected by treatment. We detected a correlation of 0.62 between phosphorylation state of S6K1 and total eIF2Bε abundance, and a correlation of 0.58 between phosphorylation state of S6K1 and total eIF2α abundance, suggesting that mTORC1 activation may have upregulated eIF2Bε and eIF2α expression. Despite maintenance of mammary eIF2Bε and eIF2α abundances during Leu and Lys deficiencies, milk protein yield declined, suggesting that other factors are responsible for mediating effects of Lys and Leu. A deficiency of all 3 BCAA may impair milk protein yield through deactivation of mTORC1-mediated upregulation of eIF2Bε and eIF2α abundances.

Received May 14, 2015.
Accepted July 17, 2015.
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Key words: mammary gland, milk protein, translation, amino acid, branched-chain amino acids

INTRODUCTION

The dietary N consumed by lactating dairy cows that is not captured in milk or body tissues is ultimately lost into the environment. Factors that stimulate milk protein yield can reduce these environmental losses and improve the economic efficiency of milk production. Essential amino acids are stimulatory to milk protein synthesis but individual EAA differ in the size of the effect they exert on milk protein yield. Increasing postruminal supplies of Met, Lys, or His has caused milk protein yield to increase to varying degrees (Schwab et al., 1976; Vanhatalo et al., 1999; Robinson, 2010). Due to the ruminal degradation and synthesis of EAA, a portion of the variation in response to supplementation of each of these EAA may be related to the availability of other EAA for protein synthesis, such that the response to Met may be limited by Lys (Schwab et al., 1976), for example. A subtraction protocol, where single EAA are subtracted from a complete supplement instead of added to a basal diet, was devised by Storm and Ørskov (1984) for ruminants and implemented in lactating ruminants (Fraser et al., 1991; Kim et al., 1999; Bequette et al., 2000) to remove the uncertainty regarding purported limitations from EAA other than the one under investigation. Subtraction of more than one-third of the metabolizable Met, His, or Phe supply negatively affects milk protein yield (Fraser et al., 1991; Kim et al., 1999; Weekes et al., 2006; Doelman et al., 2015). These group I EAA are used by milk secretory cells for protein synthesis only and are not catabolized, whereas other EAA are catabolized in the mammary glands to provide amino groups for NEAA synthesis (Mepham, 1982). Members of this second category of EAA are considered group II AA (Mepham, 1982) and include Lys, Arg, Thr, and the branched-chain AA (**BCAA**) Leu, Ile, and Val. Subtraction of more than one-third of the metabolizable Lys supply to cows decreases milk protein yield (Weekes et al., 2006; Lapierre et al., 2009), whereas subtraction of a similar fraction of

the Arg supply has had no effect (Doepel and Lapierre, 2011; Haque et al., 2013). Branched-chain amino acid subtraction produces equivocal responses. Subtraction of 40 g/d of Leu from 135 g/d of metabolizable Leu, or 23 g/d of Val from 111 of g/d metabolizable Val decreased milk protein yield (Rulquin and Pisulewski, 2006; Haque et al., 2013), whereas subtraction of 19 g/d of Ile from 99 g/d of metabolizable Ile had no effect (Haque et al., 2013). Despite the responses to Leu and Val, subtraction of 160 g/d of all 3 BCAA from 411 g/d of BCAA had no effect on milk yield or composition (Weekes et al., 2006).

Different responses to BCAA could be related to their potency as stimulators of protein synthesis via mammalian target of rapamycin complex 1 (**mTORC1**). The cellular global rate of mRNA translation is controlled by mTORC1 through phosphorylation of ribosomal S6 kinase 1 (**S6K1**) and eukaryotic initiation factor (**eIF**) 4E-binding protein 1 (**4EBP1**) in response to stimulation by protein kinase B (**Akt**) and AA (Shimobayashi and Hall, 2014). The AMP-activated protein kinase (**AMPK**) inhibits mTORC1 to slow down protein synthesis (Gwinn et al., 2008). By these mechanisms, mTORC1 integrates intracellular well-being with external hormonal signals to decide upon an appropriate rate of global protein synthesis. The postprandial increases in muscle and liver protein synthesis in nonruminants have been attributed to mTORC1 activation (Kimball et al., 2000). Recently, we reported that infusions of a complete EAA mix or mixes lacking His, Met, or Phe stimulated mammary mTORC1 and increased abundance of the eIF2B enzyme responsible for preparing eIF2 to engage in initiation of mRNA translation (Doelman et al., 2015). This increase in eIF2B may have been due to an mTORC1-mediated stimulation of translation of eIF2B mRNA into protein (Kubica et al., 2008). The BCAA, particularly Leu, are potent stimulators of mTORC1 in muscle, liver, and mammary cells (Anthony et al., 2000; Moshel et al., 2006; Toerien et al., 2010; Appuhamy et al., 2012; Suryawan et al., 2012) so the subtraction of BCAA may produce a different mammary mTORC1 response than subtraction of other EAA. Although abomasal infusion of mixtures of all EAA except His, Met, or Phe activated mammary mTORC1, only a complete mix of all EAA stimulated milk protein yield (Doelman et al., 2015), suggesting that low plasma concentrations of the EAA missing from imbalanced infusates interfered with the milk protein response. The proposed mechanism by which single EAA deficiencies interfere with protein synthesis is through phosphorylation of the α subunit of eIF2, which then becomes an inhibitor of eIF2B (Proud, 2005). However, abomasal infusates lacking His, Met, or Phe did not affect phosphorylation of mammary eIF2α (Doelman et al., 2015).

The purpose of the current experiment was to evaluate effects of deficiencies and imbalances of group II EAA on mammary S6K1, eIF2Bε, and eIF2α abundances and milk component yields in lactating cows. Cows were fed a low-protein diet as a negative control and infused with all 10 EAA as a positive control. In an attempt to create deficiencies of group II EAA, Lys, Leu, or all 3 BCAA were subtracted from the positive control. Responses to infusion of these incomplete EAA mixtures compared with the negative control were considered effects of AA imbalances.

MATERIALS AND METHODS

Experimental Protocol and Sampling

All animal procedures were approved by the Animal Care and Use Committee at Nutreco Canada Agresearch, adhering to guidelines set forth by the Canadian Council on Animal Care (2009). Five multiparous (2.2 ± 0.4 lactations) rumen-cannulated, lactating dairy cows producing an average of 31.1 kg/d at 105 ± 12 d of lactation and 631 ± 47 kg of BW were randomly assigned to a 5 × 5 Latin square design, where each period consisted of 5 d of infusion followed by 2 d of rest. Cows were fed a TMR to provide an NE_L of 6.57 MJ/kg DM and 11.2% CP (Table 1) to meet 100% and 70% of net energy and MP requirements, respectively (NRC, 2001). Production targets were 32 kg/d of milk yield containing 1,024 g of fat and 896 g of protein. Cows were acclimated through ad libitum intake of the diet for 14 d before start of the experiment. They were fed once daily at 0700 h for the duration of the experiment at a fixed amount equal to the daily average during the last 7 d of the acclimation period. Cows were weighed 2 d before the first period and at the end of each period. Feed refusals were measured daily and feed samples were taken on a weekly basis, stored at –20°C, pooled, and subsampled for proximate analysis. Silages were monitored weekly for DM content and the TMR mix was adjusted accordingly.

Infusion lines were inserted into the abomasum via rumen cannulas 1 d before the first experimental period and checked daily for patency. A Watson-Marlow 205U/CA multi-channel peristaltic pump (Wilmington, MA) was used to abomasally infuse treatments of saline (**SAL**), all 10 EAA (EAA), and EAA without Lys (**Lys−**), Leu (**Leu−**), or the branched-chain AA (Ile, Leu, and Val; **BCAA−**). Amino acids were infused at the following rates (g/h), equivalent to their secretion in 1,000 g/d of casein according to Metcalf et al. (1996)