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Glucose supplementation stimulates peripheral branched-chain amino acid catabolism in lactating dairy cows during essential amino acid infusions

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ABSTRACT

To determine how glucose modulates protein synthesis when essential AA are in abundant supply, 5 early-lactation, rumen-fistulated Holstein dairy cows were fed a diet containing 6.95 MJ/kg of net energy for lactation and 12.4% crude protein and abomasally infused for 5 d with saline, 844 or 1,126 g/d of a complete essential AA mix, with and without the inclusion of 1,000 g/d of glucose, in a 5 × 5 Latin square design. Infusion of essential AA increased milk yield by 4.1 kg/d, milk protein by 256 g/d, milk fat by 95 g/d, and milk urea nitrogen by 70% compared with saline, with no differences between the level of essential AA infusion. The addition of glucose to essential AA infusate did not stimulate milk protein yield or concentration, but reduced milk urea nitrogen by 17% and decreased milk fat yield. Arterial concentrations of total essential AA increased 3- to 4-fold, mammary clearance decreased 61%, and mammary uptake of essential AA increased 65% in response to essential AA infusion. Arterial branched-chain AA concentrations declined 29% in response to glucose and mammary clearance increased 48%, but mammary AA uptake was unchanged. Essential AA infusion increased plasma 3-methylhistidine by 50% and reduced muscle branched-chain α-keto acid dehydrogenase kinase abundance by 14%, indicating stimulation of muscle protein turnover and branched-chain AA catabolism, respectively. Glucose had no further effect on muscle branched-chain α-keto acid dehydrogenase kinase abundance but decreased mRNA expression of branched chain aminotransferase 1. Lack of further increases in plasma 3-methylhistidine or greater stimulation of muscle branched-chain AA catabolism indicates that muscle protein degradation was unchanged with glucose but that accretion may have been stimulated. The decrease in circulating branched-chain AA concentrations and nitrogen excretion in response to glucose suggests that surplus essential AA were redirected to peripheral, extra-mammary tissues.
Key words: amino acid, glucose, protein synthesis, mammary, muscle

INTRODUCTION

Milk protein yield from dairy cows can be manipulated by both protein and energy supply, but these dietary components appear to exert different effects on whole-body metabolism and AA partitioning. It is well known that more than 50% of dietary protein may not be captured in milk protein; most of the N loss occurs at the postabsorptive level, where AA not used for milk protein are partitioned into skeletal muscle, other labile protein pools, or catabolic pathways (Hanigan et al., 1997, 1998a). Increased mammary uptake and use of AA for milk protein synthesis would improve efficiency of use, lowering ingredient cost and reducing environmental N loss. Because protein synthesis is an energy-demanding process, increasing energy supply to the mammary gland may enhance milk protein yield and efficiency of N capture. Many groups have observed positive effects of energy on lactation performance and postabsorptive N efficiency. Cows fed 16.6 and 14.6% CP diets, both with 6.7 MJ of NE_L/kg of DM, produced 220 and 160 g/d more milk protein, respectively, than cows fed 5.9 MJ/kg (Rius et al., 2010). Efficiency of conversion of absorbed N into milk N increased when glucose was added to abomasal casein infusions (Clark et al., 1977). Raggio et al. (2006a,b) stimulated milk protein synthesis with infusions of casein into the duodenum or propionate into the rumen, with a greater increase observed for casein infusion and an additive effect when the 2 were infused together. The stimulatory effect of glucose or glucose precursors on AA partitioning to the mammary glands may be mediated in part by insulin, as shown from the increased mammary blood flow and milk protein yield response to a hyperinsulinemic-euglycemic clamp (Griinari et al., 1997; Mackle et al., 2000).

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Although several studies report positive milk yield responses to glucose or glucose precursor infusions (Hurtaud et al., 2000; Rulquin et al., 2004; Raggio et al., 2006a,b), in some instances glucose did not stimulate milk protein yield (Clark et al., 1977; Vanhatalo et al., 2003a,b; Curtis et al., 2014). It is possible that EAA supply to the mammary gland limits the effect of postruminal glucose on milk protein yield. Glucose infusions have been shown to decrease plasma concentrations of EAA, primarily the branched-chain AA (**BCAA**; Hurtaud et al., 1998, 2000; Lemosquet et al., 2004). In response to propionate + casein infusion, mammary uptake of EAA increased, but during propionate infusion alone, NEAA uptake increased more than EAA uptake (Raggio et al., 2006a), probably because EAA were in short supply. Supply of energy precursors can alter the profile of mammary uptake depending on the concomitant supply of EAA available to the gland. Additionally, if insulin is mediating the protein synthetic response at the mammary glands, it is possible that variation in the insulin response to glucose results in inconsistent effects on milk protein yield (Rulquin et al., 2004). Furthermore, the reductions in plasma EAA concentrations and N excretion have been interpreted to suggest that glucose partitions AA toward extramammary tissues, such as skeletal muscle (Clark et al., 1977; Curtis et al., 2014).

The objective of this study was to determine the effects of supplemental glucose on milk protein production in lactating dairy cows during EAA infusion. A high level of AA infusion, in comparison with previous studies (Metcalf et al., 1996; Doepel and Lapierre, 2010; Galindo et al., 2011; Doelman et al., 2015a,b), was used to ensure that AA would not limit milk protein yield when glucose was infused. In previous experiments, we infused EAA in equivalent amounts found in 1 kg/d of casein into cows fed a low-protein diet, and observed an average increase of 164 g/d in milk protein yield compared with a saline control (Doelman et al., 2015a,b). In the current experiment, we applied the same infusion model with 1.5 and 2 times the EAA supply.

MATERIALS AND METHODS

Experimental Protocol and Sampling

All experimental procedures were approved by the Animal Care and Use Committee at Trouw Nutrition Agresearch, adhering to guidelines set forth by the Canadian Council on Animal Care (2009). Five rumen-fistulated, multiparous (2.4 ± 0.5 lactations; second lactation n = 3, third lactation n = 2) Holstein cows producing an average of 33.0 kg/d at 78 ± 13 DIM and 576 ± 70.3 kg of BW were randomly assigned to a 5 × 5 Latin square design in which each period consisted of a 5-d continuous abomasal infusion followed by 2 d of rest. Cows were housed in tie stalls with individual free access to water and milked twice daily at 0500 and 1600 h. A TMR (Table 1) was formulated to provide an NE_L of 6.94 MJ/kg of DM and 12% CP to meet 100 and 70% of NE_L and MP target requirements, respectively. Production targets were 32.5 kg/d of milk yield consisting of 3.8% fat and 2.8% protein. Cows were acclimated to the diet for 14 d before the start of the experiment and were fed once daily at 0700 h for the duration of the experiment at a fixed amount based on the daily average as calculated using the last 7 d of the 14-d acclimation period. Cows were weighed 2 d before the start of the experiment and at the end of each period. Feed refusals were measured daily and TMR 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 placed in the abomasum via the rumen cannula 1 d before the first experimental period and were checked daily for patency and position. Infusion treatments were 0.9% saline (**SAL**), or complete mixtures of EAA with the same profile and amount as found in 1,500 and 2,000 g of casein according to Metcalf et al. (1996), with or without the inclusion of 1 kg of glucose (**1.5EAA**, **2EAA**, **1.5+GLC**, and **2+GLC**, respectively). Treatment solutions were prepared daily in 10-L batches and were infused using

Table 1. Ingredient and chemical composition of TMR

Component	Content (% of DM)
Ingredient composition	
Corn silage	49.8
Haylage	17.0
Corn, ground	15.7
Wheat shorts	6.1
Straw, chopped	5.3
Mixed hay	2.6
Tallow	1.2
Rumen-protected soybean meal[1]	1.1
Vitamin/mineral mix[2]	1.1
Urea	0.003
Nutrient analysis	
CP	12.4
NDF	28.7
ADF	18.6
NFC	46.8
Crude fat	5.3
Ash	6.8
Ca	0.71
P	0.39
NE_L (MJ/kg)	6.94

[1]TopSoy obtained from Shur-Gain Feed Mill (St. Mary's, ON, Canada).
[2]Contained 42% limestone, 32% K, 14% Mg, 11% Ca, 0.3% vitamin E, 0.3% NaCl, 0.3% Se.