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Milk protein yield and mammary metabolism are affected by phenylalanine deficiency but not by threonine or tryptophan deficiency

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ABSTRACT

Efficient milk protein synthesis requires that the essential AA be presented to the mammary gland in the right amount and proportion to maximize protein synthesis and minimize losses. This study investigated the effects of individual AA deficiencies on cow productivity, mammary metabolism, and glucose whole-body rate of appearance. Five Holstein cows were used in a 5 × 5 Latin square design trial with 10-d periods. Treatments were abomasal infusions of (1) water (CTL); (2) complete AA mixture (TAA); (3) TAA without Phe (No-Phe); (4) TAA without Thr (No-Thr); and (5) TAA without Trp (No-Trp). Each treatment was compared with TAA. Treatment did not affect milk, fat, or lactose yields. Arterial concentrations of Phe, Thr, and Trp decreased with their respective deletions by 60, 76, and 69%. In response to the decreased arterial supply of the deleted AA, mammary plasma flow significantly increased by 55% with No-Thr but did not increase with No-Phe or No-Trp. Mammary uptake of Phe was reduced by No-Phe, accompanied by a reduced milk protein yield; uptakes of Thr and Trp were not affected by their respective deletions, and milk protein yield did not decrease with these treatments. Deletion of Phe tended to reduce its mammary uptake relative to milk output (U:O), accompanied by an increased U:O of Tyr, but deletion of Thr and Trp did not affect the U:O of the corresponding AA. Plasma urea-N concentration was lower with CTL and tended to be higher with No-Phe. Arterial concentrations and mammary uptake of acetate, β-hydroxybutyrate, glucose, and lactate were unaffected by treatment. Treatment had no effect on glucose rate of appearance at the whole-body level. Lactose output as a percentage of glucose whole-body rate of appearance was not affected by treatment. Overall, the study indicated that a deficiency of Phe negatively affected productivity and mammary metabolism but that a deficiency of Thr or Trp did not.

Key words: phenylalanine, threonine, tryptophan, mammary, glucose

INTRODUCTION

The most effective strategy to increase the efficiency of nitrogen utilization in dairy cows is to decrease the amount of N fed (Doepel et al., 2004; Kebreab et al., 2010), but this strategy can be used on farms only to the extent that it can be done without detrimental effects on milk production. Reducing CP intake, and therefore the margin of safety on protein supply, might create deficiencies in the supply of some AA, which could have a negative effect on milk and milk protein yields; hence, the increased need to properly balance AA supply with demand. Milk and milk component yields respond strongly to deletion of the EAA from an infused total AA mixture, but do not respond to deletion of the NEAA (Metcalf et al., 1996; Doepel and Lapierre, 2010). Lysine and Met are considered first- and second-limiting AA in corn-based diets (NRC, 2001). On grass silage-based diets, His was reported to be first-limiting (Varvikko et al., 1999), although this limitation might be more related to the high proportion of microbial protein in the MP supply than in grass silage per se (Lee et al., 2012). The majority of AA research in dairy cows has focused on these 3 AA plus the branched-chain AA (**BCAA**; e.g., Weekes et al., 2006); however, the other EAA also need to be supplied by the digested protein to sustain milk production. Although Phe, Thr, and Trp have received minimal attention, the importance of these AA should not be overlooked. Indeed, in a series of studies conducted in the 1970s, addition of Thr to an AA mixture increased milk yield, and addition of His, Phe, and Thr to an AA infusion increased milk protein concentration (Schwab et al., 1976). Recently, Doelman et al. (2015) reported that deletion of Phe from an AA mixture infusion decreased milk protein yield by 24%, whereas deletion of Trp had no effect compared with the complete EAA infusion.

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When additional AA are supplied postruminally, an increase in milk lactose yield occurs that is almost identical to that of milk protein yield (Lapierre et al., 2010). This suggests a close interaction between AA supply and glucose availability, utilization, or both. Indeed, when extra AA have been supplied postruminally, glucose availability at the whole-body level (measured as whole-body rate of appearance: **WBRa**) increases (Lemosquet et al., 2009; Lapierre et al., 2010). Amino acids may contribute to increased glucose WBRa through 2 mechanisms: altered absorption and gluconeogenesis. The contribution from AA to glucose availability via hepatic gluconeogenesis is estimated to vary between 12 and 20% (Reynolds et al., 2003; Lapierre et al., 2010). In cows in established lactation, the increase in glucose WBRa in response to a free AA or casein infusion could be completely accounted for by the increase in hepatic net flux of glucose (Galindo et al., 2011). On the other hand, deletion of His, Met, or Lys from an AA mixture infused abomasally increased circulating glucose concentrations in dairy cows (Weekes et al., 2006). Because deletion of the 3 AA decreased milk protein yield compared with the total AA mixture, the increased glucose concentration might reflect that AA supply not used for milk protein synthesis was used to support gluconeogenesis without a concomitant increase in utilization because lactose secretion was unaffected by treatments.

Our hypothesis was that deletion of Phe, Thr, or Trp from an AA mixture infused into the abomasum would decrease milk and milk protein yields, and concomitantly increase glucose WBRa. Therefore, the objectives of this study were to determine how milk and milk component yields change when the supply of Phe, Thr, or Trp is decreased, and to determine the relationship between these AA deficiencies and glucose availability and mammary uptake of AA and energy-yielding nutrients.

MATERIALS AND METHODS

Feeding and Treatments

The experiment was carried out at the Dairy Research and Technology Centre at the University of Alberta (Edmonton, AB, Canada). Animals were cared for in accordance with the Canadian Council on Animal Care (2009), and the Animal Care and Use Committee of the University of Alberta approved all experimental procedures. Five second-lactation Holstein cows averaging 545.8 ± 15.5 kg were used. They were housed in a tiestall barn, had free access to water, and were milked at 0400 and 1500 h. At 38 ± 1.6 DIM, 2 abomasal catheters were implanted in each cow, as described by Doepel et al. (2006), and treatments were initiated 25 d later.

The experimental design was a 5 × 5 Latin square with 10-d periods. To avoid treatment carryover effects between periods and to allow for sampling on consistent days of the week, each period was followed by 4 d in which the cows did not receive treatment. The experimental treatments were abomasal infusions of (1) water (control, **CTL**); (2) all AA supplied at a rate such that the diet plus the infusion supplied 100% of the cow's MP requirement (as determined by NRC, 2001) (**TAA**); (3) all AA without Phe (**No-Phe**); (4) all AA without Thr (**No-Thr**); or (5) all AA without Trp (**No-Trp**). The AA composition of the infusion was the same as that in milk casein (Galindo et al., 2011) except that part of the Glu was replaced with Gln due to solubility limitations (Table 1). The infusates were prepared every 2 to 3 d; AA were dissolved in 15 L of water and infused continuously via peristaltic pump at a rate of 625 mL/h.

A TMR was formulated to supply 100% of the net energy requirement and 66% of the MP requirement for a second-lactation cow weighing 550 kg and producing 40 kg of milk/d with 3.6% fat and 3.2% protein (NRC, 2001; Table 2). At the start of the study, both DMI and milk yield were lower than predicted; the NRC-predicted DMI was 22.6 kg/d but the cows were

Table 1. Daily amount (g) of AA infused abomasally into the cows

AA	TAA[1]
Ala	27.7
Arg	33.3
Asn	34.3
Asp	27.8
Cys	3.6
Gln	135.0
Glu	52.9
Gly	16.7
His	24.8
Ile	44.8
Leu	83.8
Lys	70.5
Met	26.0
Phe	46.3
Pro	95.5
Ser	51.4
Thr	39.0
Trp	10.8
Tyr	49.6
Val	51.0
Total	924.8

[1]The TAA treatment included all AA supplied at a rate such that the diet plus the infusion supplied 100% of the cow's MP requirement (as determined by NRC, 2001); for No-Phe, No-Thr, and No-Trp treatments, the respective AA was deleted from the infusate.