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Hepatic expression of aminoadipate semialdehyde synthase is unchanged by postruminal lysine supply in lactating dairy cows

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ABSTRACT

Lysine supply is potentially limiting for milk production in dairy cows. The availability of Lys to the mammary gland and other tissues is a function of the quantity of metabolizable Lys supplied and Lys catabolism by the liver. Likewise, Lys catabolism may be influenced by Lys supply. This study evaluated the effect of increased postruminal Lys supply on the expression of aminoadipate semialdehyde synthase (*AASS*, a committing step in Lys catabolism in the liver) and ornithine transcarbamoylase and argininosuccinate synthase (key urea cycle enzymes that are responsive to protein supply). Eight multiparous peak Holstein cows were used in a replicated 4 × 4 Latin square. Cows were fed a Lys-limiting ration and infused postruminally with 0, 9, 27, or 63 g/d of Lys. The study consisted of 10 d of pretreatment followed by 10 d of Lys infusion. On the last day of each period, liver and milk samples were collected for mRNA analysis, and blood samples were collected for analysis of amino acids and Lys metabolites. Milk protein percent increased by 5.9%, plasma Lys increased by 74%, and α-aminoadipic acid increased by 51% with postruminal infusion of 63 g/d Lys compared with 0 g/d. Expression of *AASS*, ornithine transcarbamoylase, and argininosuccinate synthase mRNA in liver did not differ with postruminal infusion of Lys. Milk fat globule mRNA for major milk proteins and *AASS* were not affected by Lys infusion. Postruminal infusion of Lys resulted in an 86% greater increase in *AASS* mRNA in the liver compared with mammary mRNA. These changes suggest that hepatic Lys metabolism is not responsive to Lys supply at the transcription level, and that the availability of Lys to extrahepatic tissue may be determined by hepatic Lys metabolism.

Key words: lysine, gene expression, postruminal infusion

INTRODUCTION

Postruminal infusion of Lys has been used as an experimental method of providing additional absorbable lysine to dairy cows, but it has yielded inconsistent effects for milk production and composition (Rulquin et al., 1993; Robinson, 2010). The lack of predictable response to Lys infusion suggests differences in the efficiency of postruminal Lys use. A portion of this inconsistency may be due to variations in hepatic Lys catabolism, mammary Lys extraction, conversion of Lys to milk protein, or a combination of these (Lapierre and Lobley, 2001; Lobley and Lapierre, 2003).

Hepatic Lys catabolism is catalyzed by a combination of lysine ketoglutarate reductase (*LKR*) and saccharopine dehydrogenase (*SDH*). Both enzymes are sensitive to dietary Lys supply (Chu and Hegsted, 1976; Muramatsu et al., 1984; Blemings et al., 1990; Foster et al., 1993) and reside on the same bifunctional protein complex, aminoadipic semialdehyde synthase (*AASS*) (Markovitz et al., 1984; Markovitz and Chuang, 1987; Papes et al., 1999). Previous research has shown a 3-fold increase in *LKR* activity in rat and swine with when Lys of 2.2% of the total diet is added (Chu and Hegsted, 1976; Blemings et al., 1998). Moreover, in mice, a 20% decrease in *AASS* mRNA abundance has been shown to correlate with a 50% reduction in the activity of *LKR* (Cleveland et al., 2008), indicating the value of *AASS* mRNA as a measure of hepatic capacity for Lys catabolism. A similar change in *AASS* in response to postruminal Lys supply in dairy cows could reflect a portion of the observed inefficiency in milk protein response with increased postruminal Lys supply (Guinard and Rulquin, 1994).

Hepatic extraction of Lys is less than 10% of portal-drained viscera Lys flux, while mammary tissue extracts Lys in excess relative to output, resulting in an uptake to output ratio of 1.35 ± 0.29 (Lapierre et al.,

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2003, 2005, 2012). This finding suggests limited hepatic control of Lys utilization as a potential obstacle for increasing mammary Lys supply. Furthermore, because mammary tissue utilizes an average of 86% of the mammary Lys supply for milk protein synthesis, there would appear to be little alternative metabolism of the Lys extracted by mammary tissue (Guinard and Rulquin, 1994; Lapierre et al., 2005). Determining the relative relationship for Lys utilization in liver and mammary tissue may be critical in determining whole-body AA use for protein synthesis and factors that regulate this process.

Amino acid turnover and exchange between tissues is a critical part of whole-body AA metabolism (Lapierre and Lobley, 2001). Data describing the rates and extent of Lys metabolism by liver and mammary tissue are a prerequisite for increased precision in formulating diets for lactating dairy cows based on MP requirements (Hanigan et al., 2004). There is also a need to determine physiological response to changes in Lys supply, focusing on specific changes in Lys catabolism. We hypothesize that Lys catabolism in dairy cows is responsive to postruminal Lys supply and involves changes in *AASS* expression in the liver. The objective of this study was to determine the effect of increasing postruminal Lys supply on mRNA transcripts of key genes in Lys and protein catabolism in liver and mammary tissue via milk fat globule. We determined the effects of Lys on *AASS* mRNA expression as an indicator of a gene specific to Lys catabolism and its effects on urea cycle enzymes as a general indicator of tissue response to postruminal AA supply.

MATERIALS AND METHODS

Animal Use and Handling

Eight early-lactation Holstein dairy cows, averaging 68 ± 3 DIM, were housed in individual tiestalls at the Purdue University Dairy Research and Education Center and used in a replicated 4 × 4 Latin square to evaluate the effects of postruminal Lys supply. Cows were stratified by DIM, assigned to 1 of 2 squares, and randomly assigned to a treatment sequence within the square. Each square consisted of 4 periods of 20 d, including 10 d of adjustment and 10 d of Lys infusion. The last 4 d of each period were used for data collection. Animal care and handling protocols were approved by the Purdue University Animal Care and Use Committee.

All cows were fed the same corn-based ration (Table 1) delivered as a TMR once daily, and fresh water was provided freely. The corn-based ration was formulated to meet all nutrient requirements, including essential AA except Lys, for a 560 ± 16 kg cow with a BCS of 2.4 ± 0.15 (average for cows used during trial), supplying NE_L of 32.3 ± 0.6 Mcal/d and MP of 2.22 ± 0.05 kg/d (NRC, 2001).

To achieve the target protein and Lys supply (5.5% of MP), animals were limit-fed. Feed was provided based on a DMI requirement estimated by the following equation: $DMI = [((BW^{0.75}) \times 0.0968) + (0.372 \times FCM) - 0.293] \times [1 - e^{(-1 \times 0.192 \times (WOL + 3.67))}]$, where WOL is week of lactation (NRC, 2001). Intake for each cow was adjusted weekly based on milk yield, milk composition, DIM, BCS, and BW. To avoid bias in DMI by treatment, intake was determined in a manner that was blind to treatment assignment and did not account for Lys contribution to MP or NE_L.

To assess target Lys input, we used 7.2% of MP, the model from the National Research Council (NRC, 2001). The basal diet supplied 2.24 kg/d MP, which corresponded to 0.75× the Lys requirement (NRC, 2001). Infusion of 9 g/d Lys increased this to 0.82× the Lys requirement. Infusion of 27 g/d of Lys allowed for 6.7% of MP, meeting 0.93× the Lys requirement. Infusion of 63 g/d of Lys supplied 1.15 × of Lys requirements NRC (2001).

Cows were released from tiestalls and milked twice daily in a milking parlor. Milk production and feed intake were measured daily. Milk samples were collected

Table 1. Diet ingredients and nutrient composition

Item	Basal TMR
Ingredient, % of diet DM	
Corn silage	39.5
Haylage	15.9
Grass hay	5.14
Ground corn grain	10.2
Corn gluten meal	3.21
Dried distillers grains	6.79
Soybean hulls	6.90
Supplement[1]	12.0
Nutrient,[2] % of DM	
CP	15.6 ± 0.07
RDP[3]	10.0
RUP[3]	5.6
NE_L, Mcal/kg	1.64 ± 0.01
ADF	25.4 ± 1.1
NDF	36.8 ± 1.1
Calcium	1.06 ± 0.02
Phosphorus	0.33 ± 0.01

[1]Contains: 37.0 % ground corn, 24.3% dried molasses, 7.29% calcium carbonate, 7.25% Enertia (ADM, Quincy, IL), 5.52% sodium bicarbonate, 4.92% urea, 3.31% salt, 2.87% monocalcium phosphate, 1.68% XP yeast (Diamond V, Cedar Rapids, IA), 1.61% Smartamine M (Adisseo, Alpharetta, GA), 1.59% Omnigen (Phibro Animal Health Corporation, Teaneck, NJ) 1.55% magnesium oxide, 0.94% trace mineral and vitamin pack, 0.16% vitamin E premix, and 0.05% Rumensin 80 (Elanco, Greenfield, IN).

[2]Values are presented as average of 4 samples ± SE.

[3]Calculated using NRC (2001) ration evaluator.