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Is D-methionine bioavailable to the dairy cow?

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ABSTRACT

Rumen-protected forms of Met contain an equimolar mixture of the D- and L-isomers. Only L-Met can be directly used for protein synthesis, but it is unclear how much of the D-isomer can be transformed into L-Met in ruminants. Four lactating dairy cows, with an average milk yield of 32.4 kg/d, received a basal diet (12.5% crude protein, supplying 1,718 g/d of metabolizable protein) in 12 equal meals per day plus an abomasal infusion of amino acids (590 g/d, casein profile without Met). They were used in 3 consecutive studies to determine utilization of D-Met. First, the cows each received portal vein infusions for 2 d of 5, 10, or 15 g/d of DL-Met in a Youden square. On the last day of each period, 6 arterial samples were collected at 45-min intervals. Concentrations of L- and D-Met were determined on a chiral column by gas chromatography-mass spectrometry. Portal infusion of 5, 10, and 15 g/d of DL-Met increased plasma total Met concentrations (19.7, 25.0, and 34.4 ± 0.6 μM) and the proportion of Met as D (19.4, 30.5, and 37.3 ± 0.7%). The fractional removal of D-Met was 6 to 7 times lower than the fractional removal of L-Met, with mean half-lives of 52 versus 8 min, respectively. Second, the same cows were infused for 8 h with L[methyl-$^{2}H_3$]Met at 1.3 mmol/h; at 2 h, cows received a bolus injection i.v. of D-[1-^{13}C]Met (6.8 mmol), and arterial samples were collected after 10, 20, 30, 40, 60, 90, 120, 150, 180, 240, 300, 360, 420, and 480 min. Expressed relative to L-[^{12}C]Met; that is, as tracer:tracee ratios, enrichments of plasma D-[1-^{13}C]Met and L-[1-^{13}C]Met averaged 1.77 ± 0.14 and 0.144 ± 0.026, respectively, 10 min after the bolus injection and declined exponentially thereafter. A minimum of 75 ± 3% of the D-[1-^{13}C]Met was transformed into L-[1-^{13}C]Met. Third, the cows received, in a crossover design, an abomasal infusion for 5 d of either DL-Met or L-Met (15 g/d) and, on the last day of each experimental period, blood samples were collected simultaneously from arterial, portal, hepatic, and mammary vessels. Arterial total Met concentrations were higher with DL- versus L-Met infusions (37.4 vs. 25.4 ± 0.5 μM), with 37.1 ± 5.0% as D-Met. The mammary gland did not extract any D-Met. Hepatic removal of D-Met was observed, but was numerically lower than the fractional extraction of L-Met. In conclusion, much of the D-Met is transformed into L-Met by the dairy cow but at a slow rate. No uptake of D-Met occurs across the mammary gland but L-Met synthesized from the D-isomer elsewhere in the body can be utilized for milk protein synthesis.
Key words: D-methionine, L-methionine, stable isotope, mammary gland

INTRODUCTION

Over the last 2 decades, special attention has been devoted to Lys and Met requirements for lactating dairy cows because both AA are recognized as often being first-limiting in the rations fed under intensive North American or European systems (Rulquin et al., 1993; Schwab, 1996). To overcome Met deficiency, commercial supplements of rumen-protected Met have been developed (see reviews: Patton, 2010; Robinson, 2010). The chemical synthesis involved results in a racemic mixture of the D- and L-enantiomers of Met. As with other AA, only the L-isomer of Met can be incorporated into mammalian proteins. Therefore, the bioavailability of D-Met depends on the rate of transformation into L-Met. Such conversion in mammals involves D-amino acid oxidase that deaminates D-Met to yield the oxo-(keto-) acid, 2-oxo-4-methylthiobutanoate. This can then be reaminated to the L-form (Friedman and Gumbmann, 1989).

The bioavailability of D-Met has been extensively studied in many species (see Lewis and Baker, 1995), and rats, chicks, pigs, rabbits, and dogs all demonstrate good conversion to L-Met when D-Met is administered by either oral or i.v. routes, although this is not the case with primates (Stegink, 1983; Lewis and Baker, 1995). For ruminants, data are scarce and somewhat equivocal. For example, in sheep, lower utilization of D-Met than L-Met has been reported for both N and S retention (Doyle, 1981) although they appear equally effective for

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support of wool growth (Doyle, 1981; Reis et al., 1989). In growing cattle, D- and L-Met produced similar increases in N retention (Campbell et al., 1996) although this tended to be lower with DL-Met infusion compared with an equimolar dose of L-Met (Titgemeyer and Merchen, 1990). In both these bovine studies, plasma total Met concentrations (D plus L) were substantially greater when either D- or DL-Met was provided compared with similar supplementation of the L-enantiomer. Indeed, measurement of the 2 enantiomers, through use of a chiral column, demonstrated unequivocally that feeding rumen-protected Met to dairy cows led to accumulation of D-Met in the plasma (Lobley et al., 2001). In most studies, the effectiveness of D-Met has been assessed through biological responses under conditions where the anabolic improvement can be achieved by either limited conversion of D-Met to the L-isomer or through direct use of the L-Met present in the racemic mixture provided. Such observations, coupled with preliminary data from dairy cows that indicated that the mammary gland did not extract D-Met (H. Lapierre and G. E. Lobley, unpublished results), raised questions about the true availability of the D-Met for milk protein synthesis in dairy cows.

The first hypothesis investigated was that although D-Met is converted to the L-isomer in dairy cows, this occurs at a slower rate than removal of L-Met and leads to accumulation of D-Met in plasma. A second hypothesis was that a proportion of the removal of D-Met involved conversion to L-Met. The third hypothesis was that conversion of D- to L-Met does not occur in the mammary gland, so that any benefits from D-Met supplementation for milk protein synthesis would rely on metabolism to L-Met in other tissues, such as liver. These hypotheses were tested in multi-catheterized cows, with stable isotopes and chiral column analysis, to quantify metabolism of D-Met to L-Met at levels of supplementation provided under normal husbandry practice.

MATERIALS AND METHODS

Animals and Treatments

Four multiparous Holstein cows, averaging (±SEM) 662 ± 21 kg of BW and 143 ± 10 DIM at the beginning of the experiment, were used in 3 studies. Cows had been surgically implanted with abomasal catheters (Doepel et al., 2006) and with chronic indwelling catheters in the mesenteric, portal, and hepatic veins plus the caudal aorta via a mesenteric artery (Huntington et al., 1989), at least 6 mo before the initiation of the project. The right carotid artery was surgically raised to a subcutaneous position to allow access to arterial blood if the aorta catheter lost patency.

Throughout the studies, milk production averaged 32.4 ± 2.5 kg/d at 3.02 ± 0.10% CP and 4.51 ± 0.31% fat from a DMI of 20.8 ± 0.5 kg/d. Cows were fed a fixed intake of a single diet (Tables 1 and 2) at a DMI corresponding to 97% of ad libitum intake measured the week before initiation of the project. This supplied 101% of net energy requirement but only 80% of requirement for MP (NRC, 2001). In addition, throughout the 3 studies, cows received an abomasal infusion (Table 3) of an AA mixture based on casein profile excluding Met but with an adjustment to raise the proportion of essential AA to 50% of the AA infused. Due to solubility constraints, Tyr was replaced by Phe and part of Glu was replaced by Gln. The AA were dissolved in hot water as batches every 2 or 3 d. The AA infusions were administered continuously at 6 L/d with a peristaltic pump and increased the MP supply (excluding Met) to 108% of requirements (NRC, 2001). The TMR was fed in 12 equal meals per day delivered at 2-h intervals by automatic feeders (Ankom, Fairport, NY). Orts, when present, were recorded and sampled daily. Moisture content of the silages was determined weekly and was used to make ration adjustments to ensure constant delivery of DM. Cows were given free access to fresh water. Cows were milked twice a day, at 0730 and 1930 h, and milk yield was recorded at each milking. Milk was sampled at each milking on the last 2 d of each experimental period of the third study. The experimental protocol was approved by the Institutional Committee for Animal Care of the Lennoxville Research Centre and animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Study 1. The 4 cows were used in a 3 × 3 Youden square design, with 3 treatments and 3 experimental periods. Each period lasted 2 d and the treatments involved portal infusion of DL-Met (50.3% L; Sigma, St.

Table 1. Ingredient and nutrient composition of the TMR fed to the cows through the study

Item	Amount
Ingredient (% of DM)	
Corn silage	40.4
High moisture corn	25.2
Grass hay	21.3
Roasted soybeans	7.1
Urea	0.3
Ca soap of fatty acids[1]	1.8
Mineral and vitamin premix	3.9
Estimations from NRC (2001)[2]	
NE_L (Mcal/d)	35.7
CP (% DM basis)	12.5
MP (g/d)	1,718

[1]Megalac, Church & Dwight Co. Inc. (Princeton, NJ).
[2]Estimated from the measured DMI through the project.