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Short communication: Short-term intravenous amino acid infusions as a method to detect limiting amino acids in dairy cattle diets

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ABSTRACT

We hypothesized that the addition of limiting AA increases dry matter intake (DMI) by reducing anaplerosis and hepatic oxidation. Accordingly, the objective of this work was to examine the effects of short-term intravenous infusions of Met, Lys, and His (which are considered the most limiting AA) on DMI as a method to detect whether specific AA are limiting in dairy cow diets. We conducted 4 experiments using Holstein cows in the immediate postpartum period to address this objective. The first experiment used 4 cows 6 to 10 d postpartum (PP) in a 4 × 4 Latin square design with 1-d periods including 12 h for infusions and 12 h for recovery. Treatments were continuous infusions of 5 (low), 10 (medium), or 15% (high) of the calculated requirement of metabolizable Met, Lys, and His or 0.9% saline (control, CONT). In the second and third experiments, 8 cows (4–12 d PP) were divided into 2 groups of 4 cows, and each group received a different diet formulated to either be low in Lys (experiment 2) or Met (experiment 3). Each experiment was a crossover design with two 1-d periods with 12-h infusions (continuous) and 12 h for recovery. Treatments were 15% of the calculated requirement of metabolizable Met, Lys, and His (high), or 0.9% saline (CONT). In the fourth experiment, 5 cows (4–14 d PP) were used in a 5 × 5 Latin square design. Periods were 2 d in which treatments were continuously infused for the first 46 h. Treatments were 0.9% saline (CONT), all (Lys, Met, and His), LM (Lys and Met), LH (Lys and His), and MH (Met and His); dosages were equal to the estimated shortage in each specific AA. In each experiment, feed intake was recorded by a computerized data acquisition system, milk yield was recorded, and milk composition was analyzed for fat, protein, lactose, and milk urea nitrogen (MUN) concentrations. Treatments did not affect DMI or yield of milk or milk components in the first experiment. In the second experiment, AA treatment increased protein percentage and reduced lactose percentage but had no effect on protein and lactose yields or DMI. In the third experiment, the AA treatment tended to increase yields of milk, lactose, and protein as well as MUN concentration but did not affect DMI. In the fourth experiment, no effects were detected for DMI and milk yield, whereas the all, LH, and LM treatments reduced milk lactose concentration compared with CONT, and MH increased MUN concentration compared with CONT and other treatments. These results failed to provide support for our hypothesis that short-term addition of these potentially limiting AA will increase DMI. This may be due to our hypothesis being inaccurate or to other factors; other limiting AA could have prevented the effects of Lys, Met, and His infusions or the infusion periods could have been too short to induce a response in DMI. Accordingly, short-term infusion of AA is probably not a sensitive method to detect limiting AA in dairy cow diets.

Key words: limiting amino acid, hepatic oxidation, dry matter intake

Short Communication

An adequate supply of AA is critical to maximize milk yield of dairy cows (NRC, 2001). Metabolizable AA are provided by RUP, microbial protein, and endogenous protein and are essential for the synthesis of tissue and milk proteins (NRC, 2001). To a lesser extent, some AA can also be used as precursors for gluconeogenesis, and all can be oxidized for energy (Lobley, 1992; NRC, 2001; Larsen and Kristensen, 2013). Excess AA are expected to increase AA oxidation (Lobley, 1992), and accordingly, a more balanced supply of EAA with respect to their utilization is expected to reduce excess AA, improve the efficiency of protein utilization, and reduce nitrogen excretion.

Among the 10 EAA (NRC, 2001), Met, Lys, and His have generally been identified as the most limiting AA (Griel et al., 1968; Broderick et al., 1974; King et al., 1990; Vanhatalo et al., 1999; Lee et al., 2012; Osorio et al., 2013). However, experiments have had

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contradictory results; for example, experiments that supplemented rumen protected Met and Lys showed inconsistent results, ranging from 14.3 to 17.3% increase in DMI and 12% increase in milk yield (Osorio et al., 2013) to a 3.7% decrease in DMI and no effect on milk yield (Socha et al., 2005). Meta-analyses showed that the effect of rumen protected Met on DMI and milk yield is complex and dependent upon the chemical form of the Met and the main forage source of the diet (Patton, 2010; Zanton et al., 2014). Moreover, production response to supplementation of the most limiting AA is dependent upon the next most limiting AA.

Most of the experiments that supplemented AA were conducted in early or mid lactation when gut fill likely dominates control of feed intake (Allen, 2014), likely lessening effects of the supplemental AA on feed intake and productive performance. Alternatively, according to the hepatic oxidation theory (**HOT**) of the control of feed intake (Allen et al., 2009), feed intake during the postpartum (**PP**) period is likely controlled by signals from the liver to the brain via vagal afferents affected by hepatic oxidation of fuels. According to the HOT, excess AA and AA imbalances will result in greater entry of metabolites to the tricarboxylic acid cycle (anaplerosis), stimulating oxidation of acetyl CoA and suppressing feed intake (Allen, 2014). In agreement with that, a recent meta-analysis by Martineau et al. (2016) showed that abomasal infusion of casein to cows in a positive MP balance, which is likely to increase the oxidation of excess AA, reduced DMI, whereas casein infusion to cows in a negative MP balance, which is expected to result in less AA excess and oxidation, increased DMI. The control of DMI is likely dominated by a signal related to hepatic oxidation for cows in the PP period that are in a lipolytic state, and anaplerosis of the tricarboxylic acid cycle by infusion of propionic acid reduced feeding within the first few hours (Allen, 2014). Therefore, we hypothesize that infusion of presumably limiting AA (His, Met, and Lys) to cows in the early PP period will decrease anaplerosis and hepatic oxidation, allowing greater DMI, and infusing AA and measuring the response in DMI can serve as a rapid method to detect whether they are limiting or not.

We performed 4 experiments in which AA were infused intravenously and the responses in DMI, milk yield, and milk components were examined. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (East Lansing). For all experiments, cows were fitted with a single jugular catheter (left or right jugular vein) 2 to 4 d before infusion, and cows were housed in tie-stalls at the Michigan State University dairy facility, fed a TMR once daily at 120% of expected intake, and milked twice daily in the milking parlor. Feed offered and refused were recorded and sampled daily throughout the experiment for determination of nutrient content (Table 1), and the amounts of feed offered and refused were weighed.

In experiment 1, four multiparous Holstein cows [6–10 DIM; 2 $\leq$ parity $\leq$ 3; 305-d mature-equivalent milk yield (**305ME**) = 14,290 $\pm$ 2,060 kg] were used in a 4 $\times$ 4 Latin square experiment balanced for carryover effects. Cows were randomly assigned to treatment sequence and received a ration (Table 1) formulated to be similar to the diet reported by Osorio et al. (2013). Periods were 24 h, beginning at the conditioned meal after feeding and included 12 h for infusions and 12 h for recovery. Cows were blocked from feed for 2 h before the beginning of each infusion. Treatments were continuous jugular infusions (1 L/period) of solutions containing Lys, Met, and His (Ajinomoto USA, Raleigh, NC): (1) low: 5% of the assumed daily metabolizable amount: 2.01 g of Met, 2.01 g of His, and 6.03 g of Lys per L, dissolved in saline; (2) medium: double the dosages of the low treatment; (3) high: triple the dosages of the low treatment; and (4) control (**CONT**): 0.9% saline.

In experiments 2 and 3, eight multiparous Holstein cows (4–12 DIM) were divided into 2 groups of 4 cows and each group was offered a ration (Table 1) formulated to either be low in Lys (experiment 2; cows were 4–11 DIM; 1 $\leq$ parity $\leq$ 2; 305ME = 12,660 $\pm$ 2,380 kg) or Met (experiment 3; cows were 10–12 DIM; 2 $\leq$ parity $\leq$ 4; 305ME = 11,800 $\pm$ 1,610 kg). After the fourth cow assigned to a group calved, all 4 cows were fed the experimental diet for at least 3 d before the experiment began. Each experiment was a crossover design with 2 1-d periods with 12-h infusions and 12 h for recovery following infusions. In both experiments, cows were randomly assigned to a treatment sequence. Treatments were continuous jugular infusions (1 L/period) of 0.9% saline (CONT) or 6.6 g of Met, 6.6 g of His, and 19.8 g of Lys (Ajinomoto USA) per L, dissolved in saline. The AA dosages were calculated to be 15% of the calculated requirement for metabolizable Met, Lys, and His in both diets.

In experiment 4, five multiparous Holstein cows (4–16 DIM; 1 $\leq$ parity $\leq$ 3; 305ME = 13,210 $\pm$ 2,150 kg) were used in a 5 $\times$ 5 Latin square design. The experimental ration (Table 1) was lower in CP than the previous experiments and the infusion periods were longer. Periods were 48 h, beginning at the conditioned meal and included a 46-h infusion period. Cows were blocked from feed for 2 h before the beginning of infusions. Cows were randomly assigned to treatment sequences and treatments were continuous jugular infusions (1 L/d) of Met, Lys, and His (Ajinomoto USA): (1) all: 62.7, 22.2, and 22.2 g/L of Lys, Met, and His, respectively, dissolved in saline; (2) 62.7 and 22.2 g/L of Lys and