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 cross-over 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; $2 \leq \text{parity} \leq 3$; 305-d mature-equivalent milk yield (**305ME**) = $14,290 \pm 2,060$ kg] were used in a 4×4 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; $1 \leq \text{parity} \leq 2$; 305ME = $12,660 \pm 2,380$ kg) or Met (experiment 3; cows were 10–12 DIM; $2 \leq \text{parity} \leq 4$; 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; $1 \leq \text{parity} \leq 3$; 305ME = $13,210 \pm 2,150$ kg) were used in a 5×5 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

Met, dissolved in saline (**LM**); (3) 62.7 and 22.2 g/L of Lys and His, dissolved in saline (**LH**); (4) 22.2 and 22.2 g/L of Met and His, dissolved in saline (**MH**); and (5) CONT (0.9% saline). Met and Lys dosages were equal to the assumed shortage in metabolizable Met and Lys according to NRC (2001), reported in Table 1. The dose of His could not be similarly calculated using NRC (2001) formulas, and therefore we used a dose equal to that of Met.

In all experiments, infusion solutions were administered using previously calibrated Baxter Flo-Gard 6201 peristaltic infusion pumps (Baxter Medical Products,

Deerfield, IL). Feed intake was monitored by a computerized data acquisition system (Dado and Allen, 1993) and the results were validated by daily measurements of feed offered and refused, which yielded DMI values within 0.3-kg range of the computerized data for all measurements. Milk yield was recorded and milk samples collected at each milking and stored with preservative at 4°C for component analysis. Samples of TMR and all diet ingredients were collected daily during all experiments and stored in plastic bags at -20°C until processed. Feed and TMR samples were processed and analyzed for DM, NDF, CP, and starch contents similar

Table 1. Ingredient composition of the experimental rations

Item	Experiment			
	1	2	3	4
Ingredient (% of DM)				
Corn silage	33.3	34.0	39.6	34.4
Alfalfa silage	6.7	8.5	—	8.5
Alfalfa hay	4.0	11.3	11.3	12.5
Wheat straw	4.0	—	—	—
Ground corn	20.0	14.1	11.3	18.8
Soybean meal	9.0	2.5	21.5	14.5
SoyPlus	5.3	—	—	—
Corn gluten feed	—	19.8	—	—
Corn gluten meal	—	5.7	—	—
Corn distillers grains	8.3	—	—	—
Blood meal	0.3	—	—	—
Urea	0.2	—	—	—
Cottonseed	3.3	—	—	—
Soybean hulls	4.0	—	12.1	7.0
Mineral-vitamin mix ¹	1.5	2.0	2.0	2.0
Limestone	—	1.1	1.1	1.1
Sodium bicarbonate	—	1.1	1.1	1.1
Analyzed nutrient composition				
DM (%)	58.0	61.0	60.9	53.6
Starch (% of DM)	27.0	24.2	20.8	24.3
NDF (% of DM)	33.3	32.6	33.4	32.0
Forage NDF (% of DM)	19.3	23.2	22.1	22.1
CP (% of DM)	17.3	17.6	18.3	16.8
Estimated nutrient composition ²				
NE _L (Mcal/kg of DM)	1.54	1.54	1.54	1.54
RDP (% of DM)	11.2	10.4	11.2	10.6
RUP (% of DM)	6.1	6.5	5.88	5.4
RDP supplied (g/d)	1,674	1,674	1,792	1,683
RDP balance (g/d)	318	268	322	218
RUP supplied (g/d)	912	1,039	943	857
RUP required (g/d)	1,896	1,887	1,837	1,855
RUP balance (g/d)	-984	-848	-894	-1,002
MP supplied (g/d)	1,678	1,828	1,810	1,692
MP balance (g/d)	-744	-626	-640	-758
Lys:Met	3.00	2.37	3.21	3.07
Lys (% of MP)	6.4	5.4	6.7	6.6
MP-Lys (g/d)	107	99	121	112
MP-Lys balance (g/d)	-13	-33	-9	-65
Met (% of MP)	2.1	2.3	2.1	2.2
MP-Met (g/d)	35	42	38	36
MP-Met balance (g/d)	-5	-2	-5	-22

¹Mineral and vitamin mix contained 25.6% NaCl, 10.0% Ca, 2.0% Mg, 2.0% P, 30 mg/kg of Co, 504 mg/kg of Cu, 20 mg/kg of I, 2,220 mg/kg of Fe, 2,083 mg/kg of Mn, 15 mg/kg of Se, 2,025 mg/kg of Zn, 300 kIU/kg of vitamin A, 50 kIU/kg of vitamin D, and 1,500 kIU/kg of vitamin E.

²Requirements were determined from estimated DMI and milk yield according to NRC (2001).

Table 2. Dry matter intake, milk yield, and milk components for experiment 1¹

Parameter	Unit	Period	Control	Low	Medium	High	SEM	P-value
DMI	kg	Infusion ²	12.0	11.9	11.9	11.8	1.5	0.95
	kg	Daily	17.7	17.1	17.2	17.3	2.0	0.92
Milk yield	kg	PM milking ³	27.2	28.4	28.6	28.6	1.1	0.72
	kg	Daily	46.1	46.9	47.6	47.3	2.1	0.84
Fat	%	PM milking	4.73	4.63	4.96	4.77	0.18	0.41
	kg	PM milking	1.29	1.31	1.41	1.36	0.08	0.46
Protein	%	PM milking	3.51	3.58	3.69	3.59	0.16	0.55
	kg	PM milking	0.96	1.02	1.06	1.02	0.09	0.18
Lactose	%	PM milking	4.72	4.67	4.65	4.65	0.08	0.73
	kg	PM milking	1.29	1.33	1.33	1.34	0.11	0.92
MUN	mg/dL	PM milking	9.53	9.58	8.83	9.33	0.73	0.62

¹Treatments were infusions of saline (control) or 3 dosages of Lys, Met, and His: 5% (low), 10% (medium), and 15% (high) of the assumed metabolizable requirement for each of the 3 AA.

²12 h.

³The PM milking occurred at the end of the 12-h infusion period.

to Yair and Allen (2017). Individual milk samples were analyzed for fat, true protein, and lactose concentration by the NorthStar Michigan Lab (Grand Ledge, MI). Statistical analysis of data for all experiments was conducted using the Fit Model procedure of JMP (version 12.1, SAS Institute Inc., Cary, NC). For all experiments, cow was included as a random effect and treatment and period were fixed effects. Main effects were declared significant at $P \leq 0.05$, and tendencies at $P \leq 0.10$.

In the dose-response experiment (experiment 1), no statistical differences were found for any response variable measured (Table 2). Moreover, no linear, quadratic, or cubic effects were detected in experiment 1. To better identify statistical effects of AA infusion, a different experimental design was used in the next experiments (2 and 3) in which only one dosage was infused to more cows that were offered diets planned to be deficient in Met or Lys. In experiment 2, treat-

ment did not affect DMI or milk yield (Table 3) but reduced lactose concentration by 1.7% ($P = 0.02$) and increased milk protein concentration by 5.0% ($P = 0.03$), with no effect on protein and lactose yields. In experiment 3, treatment tended to increased milk yield by 7.3% ($P = 0.06$, Table 3) as well as protein yield (5.6%, $P = 0.08$), lactose yield (7.9%, $P = 0.08$), and MUN concentration (7.8%, $P = 0.08$). Because no effects were found on DMI so far, the next experiment was designed differently. In experiment 4 cows were either given no AA, all 3 AA, or combinations of 2 AA. Unlike the previous experiments, this design can enable us to detect an effect of each of the 3 individual AA. In experiment 4, none of the treatments affected DMI or milk yield (Table 4). The all, LH, and LM treatments reduced lactose concentration by 1.4, 1.2, and 2.7%, respectively, in comparison to CONT, but had no effect on total lactose yield (Table 4) and MH increased MUN concentration by 6.4% compared with CONT.

Table 3. Dry matter intake, milk yield, and milk components for experiments 2 and 3¹

Parameter	Unit	Period	Experiment 2				Experiment 3			
			CONT	TRT	SEM	P-value	CONT	TRT	SEM	P-value
Dry matter intake	kg	Infusion ²	10.7	9.5	0.8	0.41	14.3	14.3	1.2	0.99
	kg	Daily	15.8	14.5	1.4	0.19	19.0	20.1	1.8	0.20
Milk yield	kg	PM milking ³	22.1	22.9	1.3	0.55	26.1	28.0	2.3	0.06
	kg	Daily	37.9	39.8	2.5	0.97	45.4	46.3	4.4	0.76
Milk fat	%	PM milking	4.35	4.38	0.31	0.94	4.31	4.20	0.31	0.84
	kg	PM milking	0.97	1.01	0.11	0.66	1.12	1.17	0.12	0.75
Milk protein	%	PM milking	3.18	3.34	0.12	0.03	3.38	3.44	0.05	0.50
	kg	PM milking	0.70	0.76	0.04	0.31	0.90	0.95	0.08	0.08
Milk lactose	%	PM milking	4.89	4.81	0.06	0.02	4.85	4.88	0.04	0.38
	kg	PM milking	1.08	1.10	0.05	0.72	1.27	1.37	0.12	0.08
MUN	mg/dL	PM milking	6.13	5.45	0.89	0.29	12.94	13.95	1.76	0.06

¹Treatments were infusions of saline (CONT) or a combination of Lys, Met, and His (TRT). In experiment 2, the cows were fed a ration with low Lys content, whereas in experiment 3 the cows were feed a ration with a low Met diet.

²12 h.

³The PM milking occurred at the end of the 12-h infusion period.

This work was an attempt to establish a rapid method for detecting limiting AA in dairy cow diets. However, none of the AA infusion treatments affected DMI in any of the 4 experiments. Moreover, no prevalent effects were observed of AA on any of the other examined parameters other than MUN, which was increased in response to AA infusion in experiment 3 and in the MH treatment in experiment 4. As MUN can be used to monitor N excretion (Jonker et al., 1998), it suggests that the infusions of Met, Lys, and His increased the excess and oxidation of AA rather than reducing them as we expected. Although such increased excess of AA might reduce DMI according to the HOT, this was not observed in any of the experiments. However, the excess AA given in the infusions were relatively small and likely did not increase anaplerosis significantly enough to induce a response in DMI. Moreover, it is likely that only some of the infused AA were deaminated and anaplerotic. The fact that none of the infusion treatments reduced or increased DMI suggests that the experimental model suggested in this work is probably not sensitive enough and that to affect DMI, the AA excess needs to be altered significantly.

The infused AA (Met, Lys, and His) are generally thought of as the most limiting in dairy cow diets in established lactation (Griel et al., 1968; Broderick et al., 1974; King et al., 1990; Vanhatalo et al., 1999; Lee et al., 2012; Osorio et al., 2013), but evidence also indicates that other AA are limiting. Larsen et al. (2014) suggested that Tyr, Leu, and Lys are the most limiting AA in the PP period, and Iroshan et al. (2013) suggested that Phe and Thr are limiting. Accordingly, the fact that Met, Lys, and His infusions did not affect DMI might be due to other limiting AA that prevented the effect of the infused AA.

The control of milk protein synthesis is complicated. For example, AA-induced protein synthesis is at least partially regulated by the mTOR pathway (Kim, 2009). Appuhamy et al. (2012) examined the effect of EAA removal on mTOR signaling in MAC-T cells and mammary tissue slices and found that Ile and Leu deletion reduced mTOR phosphorylation. On the other hand, His, Met, or Lys, which are considered the most limiting in dairy cow diets, had no significant effect on mTOR phosphorylation, suggesting that supplementing limiting AA alone may not be enough to induce protein synthesis. Changes in AA mobilization to the mammary gland, hormonal levels (e.g., insulin), and metabolic pathways (e.g., gluconeogenesis) also add to this complexity. Accordingly, the hypothesis of the current work, which was based on the concept that providing limiting AA will increase the AA available for milk protein production and reduce AA excess, may be too simplistic.

In all experiments, we infused 2 or 3 AA (which are considered most limiting in dairy cow diets) rather than 1 AA to prevent the effect of one limiting AA being masked by the next limiting AA. If one or more of the AA that we supplemented reduced AA excess, we expected to observe an increase in DMI and reduction in MUN concentration within the infusion period because infusion of anaplerotic metabolites to cows in the PP period reduces DMI quickly (Stocks and Allen, 2013). Osorio et al. (2013) supplemented rumen-protected Met from -21 to 30 d PP and found an increase in DMI only from 7 to 21 d PP (not earlier). In this work we infused AA for 12 or 46 h. It is possible that adaptations in hepatic or whole-body AA metabolism occur after a longer-term exposure to limiting AA. However, longer AA infusions are not compatible with our goal

Table 4. Dry matter intake, milk yield, and milk components for experiment 4¹

Parameter	Unit	Period	CONT	All	LH	LM	MH	SEM	P-value
DMI	kg	0–24 h	19.8	20.5	19.7	20.3	20.3	1.0	0.75
		24–48 h	21.1	24.8	23.4	24.5	23.7	2.8	0.58
		Total 48 h	40.9	45.3	43.1	44.8	44.0	2.7	0.48
Milk yield	kg	0–24 h	42.4	42.1	42.0	42.7	41.2	2.1	0.96
		24–48 h	42.3	41.7	43.4	44.4	43.7	2.8	0.85
		Total 48 h	84.7	83.8	85.4	86.8	84.9	4.6	0.96
Fat	%	Daily average	4.33	4.41	4.49	4.56	4.32	0.24	0.75
	kg/d	Daily average	1.84	1.85	1.95	1.92	1.82	0.13	0.48
Protein	%	Daily average	3.10	3.14	3.22	3.23	3.20	0.13	0.49
	kg/d	Daily average	1.32	1.32	1.39	1.36	1.36	0.08	0.90
Lactose	%	Daily average	4.90 ^a	4.82 ^b	4.84 ^b	4.77 ^c	4.89 ^a	0.08	0.0003
	kg/d	Daily average	2.07	2.02	2.10	2.02	2.09	0.11	0.88
MUN	mg/dL	Daily average	11.45 ^b	11.17 ^b	11.52 ^b	11.62 ^b	12.18 ^a	0.96	0.006

^{a–c}Means within a row with different superscripts differ ($P < 0.05$).

¹Treatments were infusions of saline (CONT) or a combination of Lys and Met (LM), Lys and His (LH), Met and His (MH), or Lys, Met, and His (all).

of developing a rapid method of detecting limiting AA in lactating cows. To conclude, in contrast to our hypothesis, short-term infusion of potentially limiting AA did not increase DMI, suggesting that infusion of AA is probably not a sensitive method to detect limiting AA.

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REFERENCES

- Allen, M. S. 2014. Drives and limits to feed intake in ruminants. *Anim. Prod. Sci.* 54:1513–1524.
- Allen, M. S., B. J. Bradford, and M. Oba. 2009. Board Invited Review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. *J. Anim. Sci.* 87:3317–3334.
- Appuhamy, J. A. D. R. N., N. A. Knoebel, W. A. D. Nayananjali, J. Escobar, and M. D. Hanigan. 2012. Isoleucine and leucine independently regulate mTOR signaling and protein synthesis in MAC-T cells and bovine mammary tissue slices. *J. Nutr.* 142:484–491.
- Broderick, G. A., L. D. Satter, and A. E. Harper. 1974. Use of plasma amino acid concentration to identify limiting amino acids for milk production. *J. Dairy Sci.* 57:1015–1023.
- Dado, R. G., and M. S. Allen. 1993. Continuous computer acquisition of feed and water intakes, chewing, reticular motility, and ruminal pH of cattle. *J. Dairy Sci.* 76:1589–1600.
- Griell, L. C., R. A. Patton, R. D. McCarthy, and P. T. Chandler. 1968. Milk production response to feeding methionine hydroxy analog to lactating dairy cows. *J. Dairy Sci.* 51:1866–1868.
- Iroshan, I. H., H. Lapierre, and L. Doepel. 2013. The effect of a limited supply of phenylalanine, threonine, and tryptophan on milk yield and composition. Pages 115–116 in *Energy and Protein Metabolism and Nutrition in Sustainable Animal Production: 4th International Symposium on Energy and Protein Metabolism and Nutrition*. J. W. Oltjen, E. Kebreab, and H. Lapierre, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Jonker, J. S., R. A. Kohn, and R. A. Erdman. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. *J. Dairy Sci.* 81:2681–2692.
- Kim, E. 2009. Mechanisms of amino acid sensing in mTOR signaling pathway. *Nutr. Res. Pract.* 3:64–71.
- King, K. J., J. T. Huber, M. Sadik, W. G. Bergen, A. L. Grant, and V. L. King. 1990. Influence of dietary protein sources on the amino acid profiles available for digestion and metabolism in lactating cows. *J. Dairy Sci.* 73:3208–3216.
- Larsen, M., and N. B. Kristensen. 2013. Precursors for liver gluconeogenesis in periparturient dairy cows. *Animal* 7:1640–1650.
- Larsen, M., H. Lapierre, and N. B. Kristensen. 2014. Abomasal protein infusion in postpartum transition dairy cows: Effect on performance and mammary metabolism. *J. Dairy Sci.* 97:5608–5622.
- Lee, C., A. N. Hristov, T. W. Cassidy, K. S. Heyler, H. Lapierre, G. A. Varga, M. J. de Veth, R. A. Patton, and C. Parys. 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. *J. Dairy Sci.* 95:6042–6056.
- Lobley, G. E. 1992. Control of the metabolic fate of amino acids in ruminants: a review. *J. Anim. Sci.* 70:3264–3275.
- Martineau, R., D. R. Ouellet, E. Kebreab, and H. Lapierre. 2016. Casein infusion rate influences feed intake differently depending on metabolizable protein balance in dairy cows: A multilevel meta-analysis. *J. Dairy Sci.* 99:2748–2761.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th ed. Natl. Acad. Press, Washington, DC.
- Osorio, J. S., P. Ji, J. K. Drackley, D. Luchini, and J. J. Looor. 2013. Supplemental Smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. *J. Dairy Sci.* 96:6248–6263.
- Patton, R. A. 2010. Effect of rumen-protected methionine on feed intake, milk production, true milk protein concentration, and true milk protein yield, and the factors that influence these effects: A meta-analysis. *J. Dairy Sci.* 93:2105–2118.
- Socha, M. T., D. E. Putnam, B. D. Garthwaite, N. L. Whitehouse, N. A. Kierstead, C. G. Schwab, G. A. Ducharme, and J. C. Robert. 2005. Improving intestinal amino acid supply of pre- and postpartum dairy cows with rumen-protected methionine and lysine. *J. Dairy Sci.* 88:1113–1126.
- Stocks, S. E., and M. S. Allen. 2013. Hypophagic effects of propionic acid are not attenuated during a 3-day infusion in the early postpartum period in Holstein cows. *J. Dairy Sci.* 96:4615–4623.
- Vanhatalo, A., P. Huhtanen, V. Toivonen, and T. Varvikko. 1999. Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. *J. Dairy Sci.* 82:2674–2685.
- Yair, R., and M. S. Allen. 2017. The effects of fructose and phosphate infusions on dry matter intake of lactating cows. *J. Dairy Sci.* 100:2651–2659.
- Zanton, G. I., G. R. Bowman, M. Vázquez-Añón, and L. M. Rode. 2014. Meta-analysis of lactation performance in dairy cows receiving supplemental dietary methionine sources or postprandial infusion of methionine. *J. Dairy Sci.* 97:7085–7101.