Influence of supplementing a methionine derivative, *N*-acetyl-L-methionine, in dairy diets on production and ruminal fermentation by lactating cows during early to mid lactation

M. A. Fagundes,* S. Y. Yang,* J.-S. Eun,*†¹ J. O. Hall,* J. O. Moon,† and J. S. Park†

*Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan 84322

†CJ CheilJedang Research Institute of Biotechnology, Suwon, South Korea 16495

ABSTRACT

The present study investigated production responses and ruminal fermentation characteristics of lactating dairy cows when supplemented with *N*-acetyl-L-Met (NALM) as a source of rumen-protected Met in metabolizable protein (MP)-deficient (MPD) or MP-adequate diet (MPA). Eight lactating dairy cows (53 ± 10.4 d in milk, average $\pm$ standard deviation) were blocked by parity and days in milk, and the experiment was performed in a replicated 4×4 Latin square design. Within each square, cows were randomly assigned to a sequence of 4 diets during each of the four 21-d periods (14 d of treatment adaptation and 7 d of data collection and sampling). A 2×2 factorial arrangement was used; MPD or MPA was combined without or with NALM: MPD without NALM, MPD with NALM (MPD+NALM), MPA without NALM, and MPA with NALM (MPA+NALM). A NALM product was supplemented in the MPD+NALM and the MPA+NALM at 30 g/cow per d. Supplementation of NALM did not affect dry matter intake (DMI) and milk yield regardless of MP concentration. In addition, supplementing NALM resulted in a similar milk true protein concentration and yield. In contrast, NALM supplementation increased milk fat concentration and yield and 3.5% fat-corrected milk yield and tended to increase energy-corrected milk yield regardless of MP difference. Additionally, trends were observed for increased 3.5% fat-corrected milk yield/DMI and energy-corrected milk yield/DMI, and the positive effects were greater under the MPA than the MPD diet, resulting in trends toward interactions between MP and NALM. Dietary treatments had similar effects on ruminal fermentation characteristics and microbial protein yield. Plasma concentration of Met increased under the MPD but not

the MPA diet, leading to an MP $\times$ NALM interaction. Overall results in the current study suggest that NALM exerted a minor influence on ruminal metabolism, but increased milk fat concentration, resulting in increases in milk fat yield and feed efficiency. Yet, potential effects of NALM on intermediary metabolism between the gastrointestinal tract, the liver, and the mammary gland need to be explored to understand utilization efficiency for production of dairy cows.

Key words: *N*-acetyl-L-methionine, feed efficiency, lactational performance, milk fat concentration

INTRODUCTION

Dietary sources of N in lactation diets are used inefficiently by dairy cows, as they excrete substantially more N in manure over milk, which increases milk production costs and environmental N excretion (Noftsger et al., 2005). Optimizing the balance of total AA in MP has been used as the most promising means to maximize lactational performance with minimal dietary CP supply (Lee et al., 2012a). Studies have suggested that Lys and Met are the most limiting AA, especially in high-producing dairy cows fed alfalfa and corn silage-based commercial diets typically used in the United States, and optimal concentrations of these limiting AA in MP are required to increase milk protein synthesis (Schwab et al., 1976). Thus, balancing for these AA is of practical significance as an effective approach to enhance milk protein production. With an increasing economic value of milk protein, dairy producers and industries have had a strong interest in nutritional modifications such as supplementation of rumen-protected AA that will increase milk protein yield (Arriola Apelo et al., 2014a).

Considerable research has been conducted over the past 3 decades to develop technologies to protect AA from ruminal degradation, and consequently ruminally protected Met (**RPMet**) products in different chemical forms have been developed and extensively used to alleviate Met deficiencies in lactating dairy cows (Over-

Received November 12, 2017.

Accepted March 25, 2018.

¹Corresponding author: jongsu.eun@cj.net

ton et al., 1996; Patton, 2010). However, attempts to increase milk protein yield by increasing the supply of Met by addition of RPMet have given mixed results mainly due to complex inter-organ metabolisms in the rumen, the liver, and the mammary gland (Bequette et al., 2003) and substantial variations on DMI and CP concentration in feeds (Sinclair et al., 2014; Zanton et al., 2014).

N-Acetyl-L-Met (**NALM**) is a Met derivative produced via protection of L-Met α -amino group with an acetyl group. The cost of production for NALM by addition of an α -amino acetyl group to DL-Met is economically infeasible, but a cost-effective method for NALM production was recently developed (CJ CheilJedang, Seoul, South Korea) by addition of an α -amino acetyl group to L-Met. The NALM has been shown to be bioavailable and capable of replacing dietary Met in animals and humans according to Rotruck and Boggs (1975). In addition, NALM was readily incorporated into tissue proteins and supported growth of rats (Rotruck and Boggs, 1975). The NALM has also been used in foods as an approved food additive (Baxter et al., 2002). Windschitl and Stern (1988) reported that Met flowing out of continuous cultures increased with *N*-acetyl-DL-Met administration without a change in bacterial Met flow, which was attributed to greater escape (67%) of the *N*-acetyl-DL-Met from bacterial degradation. In the literature, however, no research has reported on the effects of supplementing NALM in lactation diet on production and ruminal fermentation in dairy cows.

We conducted the present study to investigate lactational performance and nutrient utilization by lactating dairy cows supplemented without or with NALM in a MP-deficient (**MPD**) or MP-adequate diet (**MPA**), according to NRC (2001). We hypothesized that increased Met supply from NALM would increase milk protein synthesis and milk yield, but the degree of the response depend upon the MP supply in the diets.

MATERIALS AND METHODS

The dairy cows used in the present study were cared for according to the Live Animal Use in Research Guidelines of the Institutional Animal Care and Use Committee at Utah State University. The study was conducted at the Caine Dairy Research Center (Wellsville, UT), Utah State University from May to July 2016.

Cows, Experimental Design, and Diets

Eight multiparous lactating Holstein cows were used during this trial. Cows began the experiment averag-

ing 53 ± 10.4 DIM, producing 39.2 ± 5.13 kg/d of milk yield, consuming 24.2 ± 3.51 kg/d of DMI, and an average BW of 696 ± 66.2 and 721 ± 57.3 kg at the beginning and the end of the experiment, respectively. The experiment was performed in a replicated 4×4 Latin square design. Within each square, cows were randomly assigned to a sequence of 4 diets during each of the four 21-d periods (14 d of treatment adaptation and 7 d of data collection and sampling). Although primary production parameters were measured in the present study, caution needs to interpret the data due to the short-term observation. Within each square, cows were randomly assigned to a sequence of 4 dietary treatments with a 2×2 factorial arrangement: MPD without NALM diet (**MPD-NALM**); MPD with NALM diet (**MPD+NALM**); MPA without NALM diet (**MPA-NALM**), and MPA with NALM (**MPA+NALM**; Table 1).

The developmental NALM product from CJ CheilJedang was used as a supplemental source of RPMet in this study. The NALM product was in powder form and contained a Met concentration of 78.0%, with 99.5% purity. The NALM product was added at a rate of 0.13% DM, and the rate was determined for cows to consume 30 g/cow per d of Met to supply 15.6 g of digestible Met to the small intestine according to the manufacturer's recommendation. This rate was also based on the requirements of digestible Met for cows fed the MPD diet. The recommendation of required digestible Lys and Met was assumed as 6.6 and 2.2% of the MP requirements, respectively (NRC, 2001). The NALM product was top-dressed onto corresponding experimental diets.

The chemical composition of alfalfa hay was 21.6, 38.9, and 27.9% DM for CP, NDF, and ADF, respectively, whereas corn silage contained 5.32, 41.5, and 23.9% DM for CP, NDF, and ADF, respectively. The diets had a forage-to-concentrate ratio of 61:39 (DM basis) on average and were typical of high-producing dairy cows in the Intermountain West (i.e., Utah, Idaho, Wyoming, Montana, and parts of Arizona and Nevada) with 44% of the forage coming from good-quality alfalfa hay. Diets were formulated based on the NRC (2001) recommendations to provide sufficient NE_L , MP, vitamins, and minerals with an estimated intake of 25.0 kg/d to produce 40 kg/d of milk with 3.5% fat and 3.0% true protein.

Cows were housed individually in tiestalls fitted with rubber mattresses covered with straw, allowing free access to water. Cows were individually fed twice daily for ad libitum intake at a level of 110% expected daily intake with 70% of allotted feed fed at 0600 h and 30% fed at 1500 h. Feed offered and refused was recorded daily, and samples were taken during the sampling

Table 1. Ingredient and chemical composition of the experimental diets¹ without or with *N*-acetyl-L-Met (NALM) at 2 different levels of MP fed to lactating Holstein dairy cows (n = 4)

Item	MPD		MPA	
	–NALM	+NALM	–NALM	+NALM
Ingredient, % of DM				
Alfalfa hay	27.0	27.0	25.8	25.7
Wheat straw	2.49	2.49	2.38	2.38
Corn silage	32.5	32.5	31.0	31.0
Corn grain (steam-flaked)	11.6	11.6	11.1	11.1
Corn grain (high-moisture)	6.17	6.17	5.90	5.89
Cottonseed, whole	4.88	4.87	4.66	4.65
Canola meal	3.50	3.50	5.68	5.68
Soybean meal	3.25	3.24	5.27	5.27
Beet pulp, shreds	4.93	4.92	4.71	4.70
NALM ²	—	0.13	—	0.13
Sodium bicarbonate	1.08	1.08	1.03	1.03
Vitamin and mineral mix ³	2.54	2.54	2.43	2.42
Chemical composition, % of DM (±SD)				
DM, %	60.9 ± 0.53	60.7 ± 0.32	61.0 ± 0.72	62.0 ± 1.50
OM	92.1 ± 0.60	91.6 ± 0.71	92.0 ± 0.84	92.0 ± 0.66
CP	15.2 ± 0.14	15.4 ± 0.21	16.1 ± 0.49	16.8 ± 0.35
RDP ⁴	9.52	9.73	9.82	10.3
RUP ⁴	5.68	5.92	6.23	6.50
NDF	37.8 ± 1.37	37.0 ± 1.88	37.4 ± 1.06	38.2 ± 0.65
ADF	23.7 ± 0.85	23.4 ± 0.88	23.4 ± 0.84	24.0 ± 0.74
Starch	17.5 ± 0.57	19.0 ± 1.91	18.9 ± 0.99	16.6 ± 0.07
Ether extract	1.66 ± 0.61	1.91 ± 0.86	1.86 ± 0.39	1.68 ± 0.73
NFC ⁵	37.4 ± 0.14	37.0 ± 0.21	36.7 ± 0.49	35.4 ± 0.35
NE _L , ⁴ Mcal/kg	1.67	1.67	1.68	1.68
Protein supply, ⁶ g/d				
RDP supply	2,342	2,443	2,494	2,616
RDP balance	–128	–27	24	146
RUP supply	1,397	1,499	1,595	1,651
RUP balance	–173	–71	25	81
MP supply	2,781	2,791	3,049	3,059
MP requirements	2,972	2,972	2,998	3,004
MP balance	–190	–181	50	55
Lys and Met balance, ⁷ g/d				
dLys requirement	184	184	201	202
dLys supplied by the diet	191	191	209	209
Lys balance	7.0	7.0	8.0	7.0
dMet requirement	61	61	67	67
dMet supplied by the diet	55	55	60	60
dMet from Met derivative	0	15.6	0	15.6
Met balance	–6.0	9.6	–7.0	8.6
dLys:dMet ⁸	3.47	2.71	3.48	2.76

¹MPD–NALM = diet deficient in MP (MPD) without NALM; MPD+NALM = MPD with NALM; MPA–NALM = diet adequate in MP (MPA) without NALM; and MPA+NALM = MPA with NALM.

²A developmental NALM product (CJ CheilJedang, Seoul, Korea) was a source of rumen-protected Met, and it contained 78.0% Met.

³Formulated to contain (per kg of DM): 226.7 mg of Se (from sodium selenite), 9,278.7 mg of Cu (from copper AA complex), 40,537.4 mg of Zn (from zinc AA complex), 38,653.4 mg of Mn (from manganese AA complex), 552.6 mg of Co (from cobalt carbonate), 1,234,585.2 IU of vitamin A, 152,808.1 IU of vitamin D, 3,815.1 IU of vitamin E, and 295 mg of Rumensin (Elanco Animal Health, Greenfield, IN).

⁴Based on tabular value (NRC, 2001).

⁵NFC = 100 – CP – NDF – ether extract – ash.

⁶All values were estimated using NRC (2001) based on actual DMI, milk yield, milk composition, and BW of the cows throughout the trial.

⁷Digestible Lys (dLys) and digestible Met (dMet) supply from the diets were estimated using NRC (2001); supply of dMet from Met derivative was estimated with Met concentration and absorption (67%) data provided by the manufacturer. Requirements of dLys and dMet were calculated as 6.6 and 2.2% (respectively) of MP requirements.

⁸Ratio of digestible Lys from diet to digestible Met from diet and NALM.

week to determine DMI. Cows were milked twice daily at 0400 and 1600 h, and milk production was recorded throughout the entire experiment.

Energy Partitioning Calculations

Energy partitioning was determined during treatment periods using data of milk yield, milk composition, and BW of experimental animals. Cows were weighed for 2 consecutive days after the a.m. milking and before the a.m. feeding at the beginning (d 1 and 2) and end (d 20 and 21) of each period. These weights were used to calculate the mean BW of cows for each experimental period. Energy used for maintenance was calculated as $BW^{0.75} \times 0.08$ (NRC, 2001). Energy of BW change was assumed to be 5.114 Mcal/kg of gain or 4.924 Mcal/kg of loss (NRC, 2001). Milk energy was calculated as $(0.0929 \times \text{milk fat concentration}) + (0.0563 \times \text{milk true protein concentration}) + (0.0395 \times \text{milk lactose concentration})$ (NRC, 2001). Estimated NE_L value was calculated by total net energy utilization (maintenance, BW gain, and milk) divided by DMI (Neal et al., 2014).

Feed and Milk Samplings and Analyses

Samples of alfalfa hay and corn silage were taken weekly to determine DM, and diets were adjusted accordingly for change in DM concentration. Samples were composited by month, ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Swedesboro, NJ), and stored for chemical analysis. Samples of TMR and orts were collected from individual cows on d 15 to 21, composited, dried at 60°C for 48 h, and ground as previously described. The DM concentrations of samples were used to calculate intakes of DM and nutrients.

Analytical DM concentration of samples was determined by oven drying overnight at 105°C, and OM was determined by ashing at 550°C for 5 h (AOAC International, 2000; method 942.05). Concentration of CP was determined using an automated N combustion analyzer (Elementar, Analysensysteme GmbH, Hanau, Germany; AOAC International, 2000; method 968.06). Concentrations of NDF and ADF were sequentially determined using a fiber analyzer (200/220, Ankom Technology, Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination and pretreated with heat-stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich, St. Louis, MO). Ether extract was measured using a fat analyzer

(XT20, Ankom Technology; AOAC International, 2000; method 2003.05). In addition, samples of TMR were analyzed for starch by Dairyland Laboratories Inc. (Arcadia, WI) according to Knudsen (1997).

Milk was sampled for 2 consecutive days (d 15 and 16) during the a.m. and p.m. milkings each period. Individual milk samples were analyzed by the Rocky Mountain DHIA Laboratory (Providence, UT) for fat, true protein, lactose, and MUN. Milk composition was expressed on weighted milk yield of a.m. and p.m. samples. Milk fat and protein yields were calculated by multiplying milk yield from the respective day by fat and true protein concentration of the milk from an individual cow.

Blood and Urine Samplings and Analyses

Blood samples were collected immediately before the morning feeding as well as at 1000 and 1400 h into 10-mL plasma vacuum tubes from the coccygeal artery or vein on d 19 and 20 in each period. Blood samples were centrifuged at $2,300 \times g$ for 20 min at 4°C, and plasma was collected and stored at -40°C for subsequent AA analysis. The plasma samples were prepared for AA analysis using the EZ:faast GC-FID Free (Physiological) Amino Acid Analysis Kit (Phenomenex Inc., Torrance, CA). Concentrations of plasma free AA were determined in accordance with the user manual provided with the kit. Extraction of free AA from the plasma consisted of a combination of solid-phase extraction, derivatization, and liquid/liquid extraction. The organic phase containing the AA in question were analyzed using a GC (model 5890, Hewlett-Packard Labs, Avondale, PA) with a capillary column (30 m $\times$ 0.32 mm i.d., 1- μ m phase thickness, Zebron ZB-FAAP, Phenomenex Inc.) and flame ionization detection.

On d 15 to 17, spot urine samples were collected from each cow at 0600 and 1800 h for a total of 6 samples per cow (Holt et al., 2013). Using 4 M HCl, urine samples were acidified to pH <4.0 and composited by cow per period. Samples were frozen and stored at -40°C. At a later date, the samples were thawed in preparation for analysis and diluted with 39 parts diluent to 1 part urine. The diluent consisted of 0.202% sodium 1-heptanesulfonate and 0.086% ammonium dihydrogen phosphate ($NH_4H_2PO_4$). The solution was brought to a pH of 2.1 using 4 M HCl. Utilization of the ratio of the urinary purine derivatives (PD) to creatinine is an accepted way to estimate the microbial protein (MCP) flow to the duodenum (Shingfield and Offer, 1998). The PD and creatinine were analyzed using an HPLC instrument (Waters Corp., Milford, MA) accord-

ing to the procedures set forth by Shingfield and Offer (1999). To estimate urine volume, creatinine was used as a marker (Valadares et al., 1999), and an average creatinine output of 28 mg/kg of BW as estimated by Whittet (2004) was assumed. Similar creatinine outputs have been reported (25 to 30 mg/kg of BW daily) (McCarthy et al., 1983; Jones et al., 1990). To estimate the relative differences in MCP production, the ratio of urinary PD (allantoin and uric acid) to creatinine was used (Shingfield and Offer, 1998), and the supply of MCP was estimated based on estimates of urinary excretion of PD according to the method of Chen et al. (1990) and Janicek et al. (2008).

Ruminal Fermentation Characteristics

Ruminal fluid samples were obtained using a Geishauser probe at 0, 3, and 6 h after the morning feeding on d 18 and 19 in each period. The fluid was collected with a solid, tube-like probe with rows of small holes on the end (Geishauser, 1993). The first 100 mL of ruminal fluid was discharged to avoid contamination from saliva, and then 10 mL was collected for analysis. The pH of the ruminal fluid was measured within 5 min of collecting the samples using a portable pH meter (Oakton pH 6, Oakton Instruments, Vernon Hills, IL). Five milliliters of the ruminal fluid was mixed with 1 mL of 1% sulfuric acid and stored frozen (-40°C) for $\text{NH}_3\text{-N}$ analysis. Concentration of $\text{NH}_3\text{-N}$ in the ruminal contents was determined as described by Rhine et al. (1998), using a plate reader (MRXe; Dynex Technologies Inc., Chantilly, VA). Another 5 mL of the ruminal fluid was collected and mixed with 1 mL of 25% metaphosphoric acid, and then stored at -40°C for VFA content determination. Ruminal VFA were separated and quantified using a GLC (model 6890 series II, Hewlett-Packard Co.) with a capillary column (30 m $\times$ 0.32-mm i.d., 1- μm phase thickness, Zebron ZB-FAAP, Phenomenex Inc.) and flame ionization detection. The oven temperature was held at 170°C for 4 min, increased to 185°C at a rate of $5^{\circ}\text{C}/\text{min}$, then increased by $3^{\circ}\text{C}/\text{min}$ to 220°C , and held at this temperature for 1 min. The injector and the detector temperatures were 225 and 250°C , respectively, and the carrier gas was helium (Eun and Beauchemin, 2007).

Statistical Analysis

All data were analyzed as a 4×4 replicated Latin square with a factorial arrangement of treatments using the Proc Mixed procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Data for intake, milk production, and energy partitioning were analyzed with

a model that accounted for effects of square, MP difference (MPD vs. MPA), supplementation of NALM ($-\text{NALM}$ vs. $+\text{NALM}$), day nested within period, and the interaction between MP, NALM, and day as fixed effects and cow within square and period within square as random effects with day included as a repeated measure. Data for ruminal pH, VFA, and $\text{NH}_3\text{-N}$ were analyzed using the model described above except that time after feeding was included as the repeated option. For each variable analyzed, 3 covariance structures (compound symmetry, autoregressive order 1, and unstructured covariance) were evaluated. The covariance structure that resulted in the lowest values for the Akaike information criterion and the Schwarz Bayesian criterion was used (Littell et al., 1998). The Kenward-Roger option was used to calculate the denominator degrees of freedom. Residual errors were used to test main effects and interactions. Differences were considered significant at $P \leq 0.05$, and trends were discussed at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

To the best of our knowledge, this is the first time that the effects of supplementing NALM in lactation dairy diets have been described. The lack of data in the literature regarding NALM led us to indirectly compare our results with data reported for 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester (**HMBi**) as well as traditional polymer-protected Met (**RPMet**) products.

A continuous culture study estimated ruminal undegradability of *N*-acetyl-DL-Met at 67% (Windschitl and Stern, 1988). Monogastric data involving young pigs supplemented with NALM revealed that no NALM was present in either portal or vena caval plasma at any sampling time (Daabees et al., 1984), which indicates hydrolysis of NALM to Met in the intestinal lumen and mucosal cells. The research reported in this paper represents initial attempts to investigate lactational performance and ruminal fermentation profiles of dairy cows fed with NALM as an alternative RPMet source.

Characteristics of Diet Composition and Treatment

Ingredients and chemical composition of experimental diets are presented in Table 1. The MPA diet provided adequate MP, whereas the MPD diet was 6% MP-deficient (-181 to -190 g/d). The MPD diet was formulated to contain less MP compared with the MPA diet by reducing the amount of dietary concentrations of canola and soybean meal in the diets. The 6% MP deficiency was chosen for the MPD based on the result

from Giallongo et al. (2016) who fed an MP-deficient diet (5–10%) supplemented with RPMet. All diets supplied NE_L in excess of the requirement (NRC, 2001). The MPA diet met or exceeded the MP, RDP, and RUP requirements, whereas the MPD diet was deficient for MP, RDP, and RUP as expected.

The NRC (2001) model suggests that Lys and Met concentrations in MP for optimal milk protein production are 7.2 and 2.4%, respectively. These concentrations are difficult to achieve without feeding very high dietary concentrations of protein. Therefore, it is recommended that the first step in balancing diets for Lys and Met is to maintain a Lys:Met in MP of 3.0 with more practical concentrations of Lys and Met in MP being 6.6 and 2.2%, respectively (Schwab et al., 2005). In this study, supplementing NALM (assuming 67% rumen undegradability) substantially improved Met status, as indicated by the reduction of the predicted (NRC, 2001) digestible Lys-to-digestible Met ratio in MP from 3.5 to 2.7 (Table 1). The digestible Lys-to-digestible Met ratio for maximum milk and milk protein yield have traditionally been reported as a 3.00:1 (NRC, 2001, Whitehouse et al., 2009), but optimal ratios as low as 2.69:1 were reported by Van Amburgh et al. (2015) when using the Cornell Net Carbohydrate and Protein System (CNCPS) model after calculating the optimum efficiency of utilization of Lys and Met using data derived by Lapierre et al. (2007) and from a meta-analysis of 40 published papers (Doepel et al., 2004).

Feed Intake, Productive Performance, and Net Energy Utilization

Intake of DM did not differ among dietary treatments, averaging 25.3 kg/d (Table 2). The MPD and the MPA diet with supplementation of NALM increased CP intake with a greater increase under the MPA diet, resulting in an MP $\times$ NALM interaction. Responses to supplementation of polymer-coated RPMet and HMBi on feed intake and productive performance in the literature have been quite variable. Kung and Rode (1996) found that rumen-protected AA supplementation did not improve DMI, which corresponds to the findings of other studies performed by Leonardi et al. (2003) and Chen et al. (2011). A limited number of studies have shown an increase in DMI with RPMet supplementation (Broderick et al., 2009; Zanton et al., 2014), whereas Ordway et al. (2009) and Zanton et al. (2014) reported decreases in DMI. Supplementation of HMBi has also increased DMI (Osorio et al., 2014) because of the potential stimulatory effect of HMBi on cellulolytic bacteria and the subsequent increase in

passage rate and DMI (Lee et al., 2015). On the other hand, Lee et al. (2012b) assessed supplementation of RPMet products to MP-deficient diets, as was explored in the current study and reported that MP-deficient diets below 15% of the NRC requirements decreased DMI. However, the authors observed that a treatment supplemented with a combination of 3 rumen-protected AA consisted of Lys (100 g/cow), Met (30 g/cow), and His (50 g/cow) increased DMI (Lee et al., 2012b). An additional study by the same group (Lee et al., 2015) found that supplementation with rumen-protected Lys and RPMet in MP-deficient diets (–256 to –305 MP g/d) did not affect DMI. In the current study, stage of lactation may have interfered with a potential effect of NALM on DMI under the MPA diet because peak DMI typically tends to lag behind peak milk in early- to mid-lactating cows that consume feed to meet energy needs.

Feeding the MPA diet increased milk yield by 2.2 kg/d relative to the MDA diet, resulting in more efficient feed utilization for milk production (1.64 vs. 1.57; Table 2). Milk yield was not affected by NALM supplementation, whereas yields of 3.5% FCM and ECM increased or tended to increase ($P = 0.06$) with NALM addition. Noftsgger et al. (2005) reported no difference in milk production when cows were supplemented with HMBi at 0.13% DM. However, St-Pierre and Sylvester (2005) showed an increase in milk yield (2.9 kg/d) to cows supplemented with HMBi at 0.15% DM. The observed production responses to supplementation of RPMet in cows fed low-protein diets (i.e., MP-deficient diets) have been, in most cases, minimal, inconsistent, or both (Arriola Apelo et al., 2014a; Sinclair et al., 2014). No negative effect on milk yield was detected by feeding a low CP diet (15%) alone or in combination with RPMet supplementation (Arriola Apelo et al., 2014b). In the current study, a 6% MP-deficient diet decreased milk yield, whereas previous research with MP-deficient diets (5–10% below MP requirement) have maintained milk yield (Lee et al., 2015; Giallongo et al., 2016). In the present study, no improvement was observed in milk yield in response to NALM supplementation in both of the MPD and the MPA diet, thus indicating that NALM was not used for milk production. Alternatively, NALM supplementation increased yields of 3.5% FCM and ECM, suggesting a potential role on fatty acid synthesis and delivery to the mammary gland possibly through a methyl donation mechanism in the liver, which will be discussed later in this paper.

A tendency was observed toward MP $\times$ NALM interaction on milk fat concentration ($P = 0.10$) because NALM supplementation increased milk fat concentration under the MPA, but not the MPD diet (Table 2).

However, milk true protein concentration was similar across the diets. Ordway et al. (2009) showed that HMBi did not increase milk fat concentration, but increased milk protein concentration in comparison to the control diet. In contrast, milk fat concentration increased with HMBi supplementation (Dalbach et al., 2011; Osorio et al., 2013). Supplementing RPMet typically results in an increase in milk protein percentage (Rulquin and Delaby, 1997; Samuelson et al., 2001; Lara et al., 2006), but one study (Benefield et al., 2009) reported a negative effect on milk protein concentration. It has been well established that Met acts as a methyl donor needed in the synthesis of milk fat (Osorio et al., 2016; Sun et al., 2016). The primary methyl carrier during bovine hepatic methyl metabolism is S-adenosyl-Met (SAM). It works to donate methyl groups for the generation of phosphatidylethanolamine, acting as phospholipids for cell membranes in the packaging of very

low density lipoprotein (VLDL; Purohit et al., 2007). Once packaged, the VLDL consist of triglycerides, apolipoproteins B and E, phospholipids, and cholesterol, and consequently Met plays a vital role in both the development of apolipoproteins and phospholipids. The VLDL are then exported from the liver to the mammary gland for milk fat synthesis (Figure 1). Thus, as a potential mode of action, NALM after its digestion and absorption may have been involved in hepatic Met metabolism pertaining to fatty acid trafficking, leading to the increased milk fat production in early to mid lactation observed in the current study.

It has been reported that aminoacylase I is present in multiple mammalian organs (i.e., small intestine, liver, and kidney; Giardina et al., 1997). The bovine liver contains the enzyme, α -N-acylamino acid hydrolase which is capable of hydrolyzing α -N-acylated AA to yield acetate and the corresponding AA (Gade and

Table 2. Intake, milk production, feed efficiency, and net energy utilization of lactating Holstein dairy cows supplemented without or with N-acetyl-L-Met (NALM) at 2 different levels of MP¹

Item	MPD		MPA		SEM	Significance of effect ²		
	–NALM	+NALM	–NALM	+NALM		MP	NALM	INT
DMI, kg/d	24.6	25.6	25.4	25.4	1.34	0.67	0.46	0.44
Yield, kg/d								
Milk	39.3	39.7	41.3	42.0	2.60	<0.01	0.48	0.81
3.5% FCM ³	39.0	39.9	40.4	43.4	2.65	0.02	0.05	0.29
ECM ⁴	38.7	39.7	40.2	42.9	2.51	0.02	0.06	0.35
Milk composition, %								
Fat	3.44	3.52	3.34	3.70	0.121	0.65	0.02	0.10
True protein	2.77	2.82	2.78	2.83	0.083	0.82	0.21	0.92
Lactose	4.84	4.87	4.88	4.85	0.049	0.65	0.97	0.26
Milk component yield, kg/d								
Fat	1.36	1.41	1.39	1.56	0.100	0.05	0.03	0.20
True protein	1.08	1.11	1.14	1.18	0.064	0.04	0.24	0.75
Lactose	1.91	1.93	2.02	2.04	0.128	<0.01	0.53	0.95
Feed efficiency								
Milk yield/DMI	1.59	1.55	1.61	1.66	0.075	0.03	0.83	0.10
3.5% FCM yield/DMI	1.58 ^b	1.57 ^b	1.57 ^b	1.72 ^a	0.083	0.06	0.09	0.06
ECM yield/DMI	1.57 ^b	1.55 ^b	1.57 ^b	1.70 ^a	0.073	0.05	0.11	0.06
BW								
Initial, kg	704	698	702	704	21.7	0.56	0.66	0.26
Mean, kg	705	705	710	713	21.2	0.03	0.74	0.60
Gain, kg/d	0.06	0.30	0.40	0.40	0.345	0.24	0.51	0.51
Net energy utilization, Mcal/d								
Maintenance	10.9	10.9	11.0	11.0	0.25	0.03	0.73	0.57
BW gain	0.24	1.16	1.55	1.55	1.322	0.24	0.51	0.51
Milk	26.2	26.1	26.8	28.5	1.67	0.03	0.20	0.16
Total ⁵	37.4	38.2	39.3	41.1	2.19	0.03	0.21	0.65
NE _L , Mcal/kg of DMI	1.49	1.55	1.55	1.60	0.074	0.13	0.18	0.94

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

¹MPD–NALM = diet deficient in MP (MPD) without NALM; MPD+NALM = MPD with NALM; MPA–NALM = diet adequate in MP (MPA) without NALM; and MPA+NALM = MPA with NALM.

²MP = level of MP in the diet (MPD vs. MPA); NALM = supplementation of NALM (–NALM vs. +NALM); and INT = interaction between MP and NALM.

³3.5% FCM = $[0.4324 \times \text{milk yield (kg/d)}] + [16.216 \times \text{fat yield (kg/d)}]$.

⁴ECM = $0.327 \times \text{milk yield (kg/d)} + 12.95 \times \text{fat yield (kg/d)} + 7.2 \times \text{protein yield (kg/d)}$.

⁵Net energy used for maintenance, BW gain, and milk.

Figure 1. Proposed mechanism of *N*-acetyl-L-Met (NALM) on its potential effects on milk fat and milk production. SAM = S-adenosyl-Met; VLDL = very low density lipoprotein (Nelson and Cox, 2013). Color version available online.

Brown, 1981). However, it is unclear how cows use the NALM into conversion of milk fat once NALM is hydrolyzed by aminoacylase I and absorbed in the small intestine as acetate and Met (Baxter et al., 2002) as illustrated in Figure 1.

Feeding the MPA diet increased milk fat yield compared with the MPD diet, and supplementing NALM further increased milk fat yield regardless of MP level (Table 2). Although feeding the MPA diet increased milk protein yield compared with the MPD diet, NALM supplementation did not influence milk protein yield. Noftsker et al. (2005) and St-Pierre and Sylvester (2005) demonstrated an increase in milk protein yield with HMBi supplementation. Supplementing HMBi and polymer-coated RPMet typically has more effect on milk protein versus milk fat yield (Lara et al., 2006; Rulquin et al., 2006; Ordway et al., 2009). This is most likely due to Met being the first-limiting EAA in lactation dairy diets, and consequently supplementation of HMBi or polymer-coated RPMet is first partitioned to improve milk protein yield in dairy cows. Contradictory effects have been reported (Phipps et al., 2008; Ordway et al., 2009) for HMBi on increased milk fat yield. Patton (2010) performed a meta-analysis on 35

studies evaluating production performance in lactating dairy cows supplemented with various RPMet products to dairy diets and found increased production of true milk protein both as a percentage (0.07%) and yield (27 g/d). However, NALM supplementation did not increase milk protein yield, and thus NALM may have been used as a source of Met in methyl donation toward VLDL production as previously discussed. Further research is needed to determine the actual contribution of Met in NALM for VLDL formation and its effects on milk production parameters.

For the diets supplemented with NALM, the increased milk fat concentration and no change in DMI resulted in a tendency toward MP $\times$ NALM interaction on 3.5% FCM yield/DMI and ECM yield/DMI ($P = 0.06$; Table 2). A 12-wk study performed by Chen et al. (2011) showed that supplementing HMBi to lactating cows averaging 143 DIM increased ECM yield/DMI. Feed efficiency is a reasonable indicator evaluated on dairy farms, as it helps measure the relative ability of cows to convert feed nutrients into milk or milk components. The increased feed efficiencies observed in the current study suggest that NALM supplementation would be economically favorable in early to mid-lactating cows,

but only when fed a diet adequate in MP concentration. However, caution should be exercised to extrapolate these benefits of NALM in the current study due to the short length of data collection, and therefore, further investigation is needed to confirm our data with a relatively long period of experiment.

No changes were seen in either BW gain or net energy utilization with supplementation of NALM in the diets (Table 2). However, feeding the MPA diet increased milk yield relative to the MDA diet, leading to an increased net energy utilization for milk. Research on RPMet supplementation on BW gain in ruminants is scarce, but some studies have reported increases in BW gain upon RPMet supplementation (Deetz et al., 1985; Oke et al., 1986). Methionine is a limiting AA in protein synthesis for growing cattle (Richardson and Hatfield, 1978), and therefore an increase in available Met can support protein deposition in the body (Loest et al., 2002). The role of Met in the formation of phospholipids through conversion of Met to SAM will also support fat deposition in growing cattle (Obeid and Herrmann, 2009). In addition, acetate, a component of NALM, is also recognized for its role as a lipogenic precursor (Bauman and Grinari, 2001). No effect on BW gain due to NALM supplementation suggests that energy was partitioned for milk fat production rather than BW gain in early to mid lactation in the present study.

Plasma AA Profiles

Among EAA, feeding the MPA diet increased plasma concentrations of Ile and Leu compared with the MPD diet, but supplementing NALM decreased those AA only under the MPA diet, resulting in an interaction between MP and NALM (Table 3). Supplementing NALM decreased plasma concentration of Val under the MPA but not the MPD diet, leading to a MP $\times$ NALM interaction. In contrast, Noftsker et al. (2005) did not find any difference in Val concentration when cows were supplemented with HMB, HMBi, or DL-Met. Similarly, St-Pierre and Sylvester (2005) supplemented a control diet with HMB, HMBi, or a combination of HMB and HMBi, and the dietary treatments had no effect on free plasma AA, including Val. Plasma concentration of Met increased under the MPD but not MPA, leading to an MP $\times$ NALM interaction. The no effect of NALM supplementation on plasma Met concentration in the MPA diet observed in this study was unexpected, and its mechanism is difficult to explain. The liver plays a central role in Met metabolism (Finkelstein, 1990); approximately 50% of Met metabolism and up to 85%

of all methylation reactions occur in the mammalian liver (Mato et al., 2002). Hepatic gene regulation serves as an important regulatory point for controlling Met metabolism (Finkelstein, 2003; Mato et al., 2008). However, effects of RPMet supplementation on genes for Met metabolism in bovine hepatocytes in vivo have not been clearly understood in lactating dairy cattle. Zhang et al. (2016) demonstrated decreased mRNA expression for genes that catalyze regeneration of Met and showed the decreased need for cellular regeneration of Met with increasing Met concentrations from 2-hydroxy-4-(methylthio)-butanoic acid (HMB) and DL-Met on primary bovine hepatocytes. The authors described that increased Met supply from HMB and DL-Met in primary bovine hepatocytes was not directed at increased glucose synthesis, probably due to other metabolic priorities for Met (Zhang et al., 2016). Likewise, in the current study it is speculated that increased Met supply from NALM supplementation in the MPA diet may have been prioritized in the bovine hepatocyte to the primary methyl donor SAM, allowing SAM to be used for increased VLDL synthesis and exportation to the mammary gland for milk fat synthesis (Figure 1).

Ruminal Fermentation Characteristics

Ruminal pH decreased in the MPD relative to the MPA diet (6.24 vs. 6.37; Table 4), but NALM supplementation did not influence ruminal pH. The mean ruminal pH from all dietary treatments were at least above 6.2, so even the effect of MP difference on overall microbial physiology may have been minor. Concentration of VFA and their compositions were in general not affected by MP level as well as NALM supplementation. Similarly, Noftsker et al. (2005) and Lee et al. (2015) reported no effects of RPMet supplementation on ruminal pH and VFA production. Concentration of $\text{NH}_3\text{-N}$ also did not differ across the dietary treatments, whereas MCP yield increased due to feeding the MPA diet compared with the MPD diet, but NALM did not affect MCP yield regardless of MP level (Table 4). Supplementation of RPMet traditionally has not had an effect on microbial production, with the exception of Met analogs such as HMBi because of its partial ruminal degradation and potential stimulatory effects on MCP synthesis (Robert et al., 2001). Similarly, Lee et al. (2015) reported increased MCP yield with polymer-coated RPMet supplementation. Although NALM is partially degraded in the rumen (approximately 33%, Figure 1), the degraded fraction of the NALM would not contribute to MCP synthesis under the experimental condition of our study. Further research is needed

Table 3. Plasma AA concentrations ($\mu\text{mol/L}$) of lactating Holstein dairy cows supplemented without or with *N*-acetyl-L-Met (NALM) at 2 different levels of MP¹

Item	MPD		MPA		SEM	Significance of effect ²		
	–NALM	+NALM	–NALM	+NALM		MP	NALM	INT
His	10.1	11.6	10.2	9.7	5.60	0.51	0.70	0.48
Ile	85.3 ^b	90.5 ^b	102 ^a	91.9 ^b	9.39	<0.01	0.35	<0.01
Leu	119 ^b	122 ^b	144 ^a	123 ^b	12.5	<0.01	0.02	<0.01
Lys	61.2	65.8	68.0	65.0	17.61	0.40	0.83	0.29
Met	18.0 ^b	19.6 ^a	18.3 ^{ab}	17.4 ^b	0.85	0.06	0.49	0.01
Phe	71.3 ^b	73.2 ^{ab}	76.4 ^a	70.6 ^b	5.23	0.44	0.24	0.02
Thr	31.6	33.1	32.1	29.0	7.38	0.25	0.59	0.13
Val	248 ^b	266 ^b	283 ^a	241 ^b	23.3	0.74	0.43	0.05
Ala	196 ^b	195 ^b	200 ^a	181 ^b	14.3	0.36	0.04	0.07
Asp	37.9	49.8	46.9	38.1	9.48	0.77	0.75	0.03
Glu	24.8	23.3	24.2	22.6	2.50	0.47	0.09	0.99
Gly	198 ^a	198 ^a	188 ^{ab}	168 ^c	20.9	<0.01	0.07	0.07
Pro	78.0 ^{ab}	75.3 ^{bc}	80.8 ^a	70.3 ^c	6.68	0.54	<0.01	0.04
Tyr	138	166	168	159	42.2	0.35	0.44	0.13
EAA ³	645 ^b	682 ^{ab}	720 ^a	648 ^b	21.6	0.34	0.42	0.01
NEAA ⁴	793	707	694	638	64.3	0.19	0.27	0.82

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

¹MPD–NALM = diet deficient in MP (MPD) without NALM; MPD+NALM = MPD with NALM; MPA–NALM = diet adequate in MP (MPA) without NALM; and MPA+NALM = MPA with NALM.

²MP = level of MP in the diet (MPD vs. MPA); NALM = supplementation of NALM (–NALM vs. +NALM); and INT = interaction between MP and NALM.

³EAA analyzed.

⁴NEAA analyzed.

Table 4. Ruminal fermentation characteristics of lactating Holstein dairy cows supplemented without or with *N*-acetyl-L-Met (NALM) at 2 different levels of MP¹

Item	MPD		MPA		SEM	Significance of effect ²		
	–NALM	+NALM	–NALM	+NALM		MP	NALM	INT
Ruminal pH	6.26	6.21	6.35	6.39	0.101	0.03	0.95	0.48
Total VFA, mM	146	154	147	144	6.6	0.53	0.68	0.39
Individual VFA, mol/100 mol								
Acetate (A)	62.4	62.0	62.0	62.4	3.79	0.96	0.93	0.26
Propionate (P)	23.3	23.7	23.2	23.3	2.99	0.60	0.65	0.80
Butyrate	10.7	10.6	11.0	10.7	0.97	0.49	0.47	0.70
Valerate	1.57	1.66	1.47	1.62	0.403	0.30	0.09	0.61
Isobutyrate	0.79	0.89	0.95	0.87	0.128	0.27	0.88	0.14
Isovalerate	1.27 ^b	1.27 ^b	1.41 ^a	1.20 ^b	0.179	0.52	0.04	0.06
A:P	2.77	2.70	2.75	2.74	0.210	0.85	0.45	0.63
NH ₃ -N, ³ mg/100 mL	9.34	9.05	9.28	9.82	1.425	0.70	0.89	0.65
MCP, ⁴ g/d	2,540	2,649	2,954	2,837	237.7	<0.01	0.96	0.16

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

¹MPD–NALM = diet deficient in MP (MPD) without NALM; MPD+NALM = MPD with NALM; MPA–NALM = diet adequate in MP (MPA) without NALM; and MPA+NALM = MPA with NALM.

²MP = level of MP in the diet (MPD vs. MPA); NALM = supplementation of NALM (–NALM vs. +NALM); and INT = interaction between MP and NALM.

³Ammonia-N.

⁴Microbial protein production (g/d) = $\{[\text{urinary total purine derivatives (allantoin + uric acid)} - (0.385 \times \text{BW}^{0.75})]/0.85\} \times 70 \times 6.25 / (0.13 \times 0.83 \times 1,000)$ (Janicek et al., 2008).

to understand the mode of action of NALM on ruminal metabolism, particularly how the aminoacylase I enzyme acts on NALM.

CONCLUSIONS

The current study investigated the production responses and ruminal fermentation profiles of early to mid-lactating dairy cows supplemented without or with a developmental NALM product in the MPD or the MPA diet. The NALM supplementation did not affect DMI, milk yield, and milk protein yield or alter ruminal fermentation characteristics in either the MPD or the MPA diet, suggesting that early- to mid-lactating dairy cows tested in this study may have other metabolic priorities for Met. However, supplementation of NALM regardless of MP difference increased both 3.5% FCM and ECM yields coupled with increased milk fat concentration, which was more impactful in the MPA diet, leading to better feed efficiencies with the MPA diet. Thus, supplementing NALM, particularly in the MPA diet, in early to mid lactation can be a good approach to formulate an optimal dairy feeding program for improving feed efficiency. Yet, the potential effects of NALM on intermediary metabolism between the gastrointestinal tract, the liver, and the mammary gland needs to be explored to understand the metabolic effects of NALM on lactational and physiological parameters of dairy cows. Overall, the results in the current study suggest that NALM exerted minor influence on ruminal metabolism, but increased milk fat concentration possibly due to accelerated fatty acid delivery to the mammary gland, resulting in sizable increases in milk fat yield and feed efficiency.

ACKNOWLEDGMENTS

This study was supported by CJ CheilJedang (Seoul, South Korea). The authors thank S. Sharp at Utah State University (Logan) for technical assistance and the staff of the Caine Dairy Research Center (Wellsville, UT) for their conscientious care of the experimental cows.

REFERENCES

- AOAC International. 2000. Official Methods of Analysis. Vol. 1 and 2. 17th ed. AOAC Int., Gaithersburg, MD.
- Arriola Apelo, S. I., A. L. Bell, K. Estes, J. Ropelewski, M. J. de Veth, and M. D. Hanigan. 2014a. Effects of reduced dietary protein and supplemental rumen-protected essential amino acids on the nitrogen efficiency of dairy cows. *J. Dairy Sci.* 97:5688–5699.
- Arriola Apelo, S. I., J. R. Knapp, and M. D. Hanigan. 2014b. Invited review: Current representation and future trends of predicting amino acid utilization in the lactating dairy cow. *J. Dairy Sci.* 97:4000–4017.
- Bauman, D. E., and J. M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: Low-fat milk syndrome. *Livest. Prod. Sci.* 70:15–29.
- Baxter, J., Y. Kim, and M. Snowden. 2002. Hydrolysis of *N*-acetyl-L-glutamine by acylase I. *J. Food Sci.* 66:1428–1433.
- Benefield, B. C., R. A. Patton, M. J. Stevenson, and T. R. Overton. 2009. Evaluation of rumen-protected methionine sources and period length on performance of lactating dairy cows within Latin squares. *J. Dairy Sci.* 92:4448–4455.
- Bequette, B. J., M. D. Hanigan, and H. Lapierre. 2003. Mammary uptake and metabolism of amino acids by lactating ruminants. Pages 347–365 in *Amino Acids in Animal Nutrition*. J. P. F. D'Mello, ed. CAB International, Wallingford, UK.
- Broderick, G. A., M. J. Stevenson, and R. A. Patton. 2009. Effect of dietary protein concentration and degradability on response to rumen-protected methionine in lactating dairy cows. *J. Dairy Sci.* 92:2719–2728.
- Chen, X. B., E. R. Ørskov, and F. D. D. Hovell. 1990. Excretion of purine derivatives by ruminants: endogenous excretion, differences between cattle and sheep. *Br. J. Nutr.* 63:121–129.
- Chen, Z. H., G. A. Broderick, N. D. Luchini, B. K. Sloan, and E. Devillard. 2011. Effect of feeding different sources of rumen-protected methionine on milk production and *N*-utilization in lactating dairy cows. *J. Dairy Sci.* 94:1978–1988.
- Daabees, T. T., D. W. Andersen, W. L. Zike, L. J. Filer Jr., and L. D. Stegink. 1984. Portal and vena caval methionine concentrations in young pigs administered L-methionine, N-acetyl-L-methionine and N-acetyl-D-methionine. *J. Nutr.* 114:1541–1547.
- Dalbach, K. F., M. Larsen, B. L. M. Raun, and N. B. Kristensen. 2011. Effects of supplementation with 2-hydroxy-4-(methylthio)-butanoic acid isopropyl ester on splanchnic amino acid metabolism and essential amino acid mobilization in postpartum transition Holstein cows. *J. Dairy Sci.* 94:3913–3927.
- Deetz, L. E., A. M. Papas, and C. H. Benton. 1985. Performance of finishing steers fed rumen-protected methionine and/or lysine. *J. Anim. Sci.* 61(Suppl. 1):486. (Abstr.)
- Doepel, L., D. Pacheco, J. J. Kennelly, M. D. Hanigan, I. F. Lopez, and H. Lapierre. 2004. Milk protein synthesis as a function of amino acid supply. *J. Dairy Sci.* 87:1279–1297.
- Eun, J. S., and K. A. Beauchemin. 2007. Enhancing in vitro degradation of alfalfa hay and corn silage using feed enzymes. *J. Dairy Sci.* 90:2839–2851.
- Finkelstein, J. D. 1990. Methionine metabolism in mammals. *J. Nutr. Biochem.* 1:228–237.
- Finkelstein, J. D. 2003. Methionine metabolism in liver diseases. *Am. J. Clin. Nutr.* 77:1094–1095.
- Gade, W., and J. L. Brown. 1981. Purification, characterization and possible function of alpha-N-acylamino acid hydrolase from bovine liver. *Biochim. Biophys. Acta* 662:86–93.
- Geishauser, T. 1993. An instrument for the collection and transfer of ruminal fluid and for the administration of water soluble drugs in adult cattle. *Bov. Pract.* 27:38–42.
- Giallongo, F., M. T. Harper, J. Oh, J. C. Lopes, H. Lapierre, R. A. Patton, C. Parys, I. Shinzato, and A. N. Hristov. 2016. Effects of rumen-protected methionine, lysine, and histidine on lactation performance of dairy cows. *J. Dairy Sci.* 99:4437–4452.
- Giardina, T., A. Biagini, F. Dalle Ore, E. Ferre, M. Reynier, and A. Puigserver. 1997. The hog intestinal mucosa acylase I: subcellular localization, isolation, kinetic studies and biological function. *Biochimie* 79:265–273.
- Holt, M. S., K. Neal, J. S. Eun, A. J. Young, J. O. Hall, and K. E. Nestor. 2013. Corn silage hybrid type and quality of alfalfa hay affect dietary nitrogen utilization by early lactating dairy cows. *J. Dairy Sci.* 96:6564–6576.
- Janicek, B. N., P. J. Kononoff, A. M. Gehman, and P. H. Doane. 2008. The effect of feeding dried distillers grains plus solubles on milk production and excretion of urinary purine derivatives. *J. Dairy Sci.* 91:3544–3553.

- Jones, S. J., D. L. Starkey, C. R. Calkins, and J. D. Crouse. 1990. Myofibrillar protein turnover in feed-restricted and realimented beef cattle. *J. Anim. Sci.* 68:2707–2715.
- Knudsen, K. E. B. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. *Anim. Feed Sci. Technol.* 67:319–338.
- Kung, L., Jr., and L. M. Rode. 1996. Amino acid metabolism in ruminants. *Anim. Feed Sci. Technol.* 59:167–172.
- Lapierre, H., G. E. Lobley, D. R. Quellet, L. Doepel, and D. A. Pacheco. 2007. Amino acid requirements for lactating dairy cows: Reconciling predictive models and biology. Pages 39–60 in *Proc. Cornell Nutr. Conf., Dept. Anim. Sci., Cornell Univ., Ithaca, NY*.
- Lara, A., G. D. Mendoza, L. Landois, R. Barcena, M. T. Sánchez-Torres, R. Rojo, J. Ayala, and S. Vega. 2006. Milk production in Holstein cows supplemented with different levels of ruminally protected methionine. *Livest. Sci.* 105:105–108.
- Lee, C., F. Giallongo, A. N. Hristov, H. Lapierre, T. W. Cassidy, K. S. Heyler, G. A. Varga, and C. Parys. 2015. Effect of dietary protein level and rumen-protected amino acid supplementation on amino acid utilization for milk protein in lactating dairy cows. *J. Dairy Sci.* 98:1885–1902.
- 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. 2012b. 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.
- Lee, C., A. N. Hristov, K. S. Heyler, T. W. Cassidy, H. Lapierre, G. A. Varga, and C. Parys. 2012a. Effects of metabolizable protein supply and amino acid supplementation on nitrogen utilization, milk production, and ammonia emissions from manure in dairy cows. *J. Dairy Sci.* 95:5253–5268.
- Leonardi, C., M. Stevenson, and L. E. Armentano. 2003. Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. *J. Dairy Sci.* 86:4033–4042.
- Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* 76:1216–1231.
- Loest, C. A., E. C. Titgemeyer, G. St-Jean, D. C. Van Metre, and J. S. Smith. 2002. Methionine as a methyl group donor in growing cattle. *J. Anim. Sci.* 80:2197–2206.
- Mato, J. M., F. J. Corrales, S. C. Lu, and M. A. Avila. 2002. S-Adenosylmethionine: A control switch that regulates liver function. *FASEB J.* 16:15–26.
- Mato, J. M., M. L. Martínez-Chantar, and S. C. Lu. 2008. Methionine metabolism and liver disease. *Annu. Rev. Nutr.* 28:273–293.
- McCarthy, F. D., W. C. Bergen, and D. R. Hawkins. 1983. Muscle protein turnover in cattle of differing genetic backgrounds as measured by urinary N'-methylhistidine excretion. *J. Nutr.* 113:2455–2463.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- Neal, K., J.-S. Eun, A. J. Young, and J. O. Hall. 2014. Feeding protein supplements in alfalfa hay-based lactation diets improves nutrient utilization, lactational performance, and feed efficiency of dairy cows. *J. Dairy Sci.* 97:7716–7728.
- Nelson, D. L., and M. M. Cox. 2013. *Lehninger Principles of Biochemistry*. 6th ed. W. H. Freeman and Company, New York, NY.
- Noftsker, S., N. R. St-Pierre, and J. T. Sylvester. 2005. Determination of rumen degradability and ruminal effects of three sources of methionine in lactating cows. *J. Dairy Sci.* 88:223–237.
- Obeid, R., and W. Herrmann. 2009. Homocysteine and lipids: S-adenosylmethionine as a key intermediate. *FEBS Lett.* 583:1215–1225.
- Oke, B. O., S. C. Loerch, and L. E. Deetz. 1986. Effects of rumen-protected methionine and lysine on ruminant performance and nutrient metabolism. *J. Anim. Sci.* 62:1101–1112.
- Ordway, R. S., S. E. Boucher, N. L. Whitehouse, C. G. Schwab, and B. K. Sloan. 2009. Effects of providing two forms of supplemental methionine to periparturient Holstein dairy cows on feed intake and lactational performance. *J. Dairy Sci.* 92:5154–5166.
- Osorio, J. S., C. B. Jacometo, Z. Zhou, D. Luchini, F. C. Cardoso, and J. J. Loor. 2016. Hepatic global DNA and peroxisome proliferator-activated receptor alpha promoter methylation are altered in periparturient dairy cows fed rumen-protected methionine. *J. Dairy Sci.* 99:234–244.
- Osorio, J. S., P. Ji, J. K. Drackley, D. Luchini, and J. J. Loor. 2013. Supplemental Smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. *J. Dairy Sci.* 96:6248–6263.
- Osorio, J. S., E. Trevisi, P. Ji, J. K. Drackley, D. Luchini, G. Bertoni, and J. J. Loor. 2014. Biomarkers of inflammation, metabolism, and oxidative stress in blood, liver, and milk reveal a better immunometabolic status in periparturient cows supplemented with Smartamine M or MetaSmart. *J. Dairy Sci.* 97:7437–7450.
- Overton, T. R., D. W. LaCount, T. M. Cicela, and J. H. Clark. 1996. Evaluation of a ruminally protected methionine product for lactating dairy cows. *J. Dairy Sci.* 79:631–638.
- 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.
- Phipps, R. H., C. K. Reynolds, D. L. Givens, A. K. Jones, P. A. Geraert, E. Devillard, and R. Bennett. 2008. Short communication: Effects of 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester on milk production and composition of lactating Holstein dairy cows. *J. Dairy Sci.* 91:4002–4005.
- Purohit, V., M. F. Abdelmalek, S. Barve, N. J. Benevenga, C. H. Halsted, N. Kaplowitz, K. K. Kharbanda, Q. Liu, S. C. Lu, C. J. McClain, C. Swanson, and S. Zakhari. 2007. Role of S-adenosylmethionine, folate, and betaine in the treatment of alcoholic liver disease: Summary of a symposium. *Am. J. Clin. Nutr.* 86:14–24.
- Rhine, E. D., G. K. Sims, R. L. Mulvaney, and E. J. Pratt. 1998. Improving the Berthelot reaction for determining ammonium in soil extracts and water. *Soil Sci. Soc. Am. J.* 62:473–480.
- Richardson, C. R., and E. E. Hatfield. 1978. The limiting amino acids in growing cattle. *J. Anim. Sci.* 46:740–745.
- Robert, J. C., C. Richard, T. D. Alfonso, N. Ballet, and E. Depres. 2001. Investigation of the site of absorption of a novel source of metabolizable methionine: 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester (HMBi). *J. Dairy Sci.* 84(Suppl. 1):35. (Abstr.)
- Rotruck, J. T., and R. W. Boggs. 1975. Comparative metabolism of L-methionine and N-acetylated derivatives of methionine. *J. Nutr.* 105:331–337.
- Rulquin, H., and L. Delaby. 1997. Effects of the energy balance of dairy cows on lactational responses to rumen-protected methionine. *J. Dairy Sci.* 80:2513–2522.
- Rulquin, H., B. Graulet, L. Delaby, and J. C. Robert. 2006. Effect of different forms of methionine on lactational performance of dairy cows. *J. Dairy Sci.* 89:4387–4394.
- Samuelson, D. J., S. K. Denise, R. Roffler, R. L. Ax, D. V. Armstrong, and D. F. Romagnolo. 2001. Response of Holstein and Brown Swiss cows fed alfalfa hay-based diets to supplemental methionine at two stages of lactation. *J. Dairy Sci.* 84:917–928.
- Schwab, C. G., R. S. Ordway, and N. L. Whitehouse. 2003. Amino acid balancing in the context of MP and RUP requirements. Pages 25–34 in *Proc. Four-State Applied Nutrition and Management Conference*. LaCrosse, WI. Midwest Plan Service, Iowa State University, Ames.
- Schwab, C. G., L. D. Satter, and A. B. Clay. 1976. Responses of lactating dairy cows to abomasal infusions of amino acids. *J. Dairy Sci.* 59:1254–1270.
- Shingfield, K. J., and N. W. Offer. 1998. Evaluation of the spot urine sampling technique to assess urinary purine derivative excretion in lactating dairy cows. *J. Anim. Sci.* 66:557–568.
- Shingfield, K. J., and N. W. Offer. 1999. Simultaneous determination of purine metabolites, creatinine and pseudouridine in ruminant urine by reversed-phase high performance liquid chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* 723:81–94.
- Sinclair, K. D., P. C. Garnsworthy, G. E. Mann, and L. A. Sinclair. 2014. Reducing dietary protein in dairy cow diets: Implications for nitrogen utilization, milk production, welfare and fertility. *Animal* 8:262–274.
- St-Pierre, N. R., and J. T. Sylvester. 2005. Effects of 2-hydroxy-4-(methylthio) butanoic acid (HMB) and its isopropyl ester on

- milk production and composition by Holstein cows. *J. Dairy Sci.* 88:2487–2497.
- Sun, F., Y. Cao, C. Cai, S. Li, C. Yu, and J. Yao. 2016. Regulation of nutritional metabolism in transition dairy cows: Energy homeostasis and health in response to post-ruminal choline and methionine. *PLoS One* 11:e0160659.
- Valadares, R. F. D., G. A. Broderick, S. C. V. Filho, and M. K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. *J. Dairy Sci.* 82:2686–2696.
- Van Amburgh, M. E., E. A. Collao-Saenz, R. J. Higgs, D. A. Ross, E. B. Recktenwald, E. Raffrenato, L. E. Chase, T. R. Overton, J. K. Mills, and A. Foskolos. 2015. The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. *J. Dairy Sci.* 98:6361–6380.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods of dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Whitehouse, N., C. Schwab, D. Luchini, T. Tylutki, and B. Sloan. 2009. Comparison of optimal lysine and methionine concentrations in metabolizable protein estimated by the NRC (2001), CPM-Dairy (v.3.0.10) and AMTS.Cattle (v.2.1.1) models. *J. Dairy Sci.* 92(Suppl. 1):103. (Abstr.)
- Whittet, K. M. 2004. Factors affecting variability in urinary creatinine and purine derivative excretion in beef cattle. MS Thesis. Department of Animal Science, University of Nebraska, Lincoln.
- Windschitl, P. M., and M. D. Stern. 1988. Influence of methionine derivatives on effluent flow of methionine from continuous culture of ruminal bacteria. *J. Anim. Sci.* 66:2937–2947.
- 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 postruminal infusion of methionine. *J. Dairy Sci.* 97:7085–7101.
- Zhang, Q., S. J. Bertics, N. D. Luchini, and H. M. White. 2016. The effect of increasing concentrations of DL-methionine and 2-hydroxy-4-(methylthio) butanoic acid on hepatic genes controlling methionine regeneration and gluconeogenesis. *J. Dairy Sci.* 99:8451–8460.