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Comparison of plasma methionine response to 3 rumen-protected methionine products in lactating Holstein dairy cows

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ABSTRACT

Objective: The primary aim of this work was to compare the plasma Met response to supplementation with 3 rumen-protected Met products. Secondary aims were to evaluate how time of sampling affected results and how pooling of samples may have affected results.

Materials and Methods: Ten multiparous Holstein cows (280 ± 73 DIM) were used in a replicated 3 × 3 Latin square design with 7-d experimental periods. Cows were fed every 8 h, and treatments consisted of supplementation of 12 g/d (4 g/feeding) of 1 of 3 rumen-protected Met (RPM) products. The products evaluated were the newly developed RPM-K and 2 existing products, RPM-S and RPM-M, with known differences in bioavailability. During d 5 to 7 of each period, blood samples were collected at 2, 4, 6, and 8 h after the morning feeding for plasma free AA analysis. Plasma Met data were analyzed using the full data set as well as mean values from individual cows for each day or each period.

Results and Discussion: Plasma Met was not different between RPM-S and RPM-K (32.7 vs. 33.0 μM, respectively; $P = 0.79$), and both were greater than RPM-M (30.1 μM; $P \leq 0.001$). Plasma Met was affected by time of sampling ($P = 0.001$), due to reduced plasma Met at 4 h (30.2 μM) than at 2, 6, and 8 h (31.9–33.0 μM). Using the daily and period mean values of plasma Met, differences observed in the full model were maintained when daily means were evaluated, but period means resulted in only a tendency for a treatment effect.

Implications and Applications: Bioavailability of RPM-K was similar to RPM-S and greater than RPM-M. Pooling samples by day within cow would have likely yielded similar results.

Key words: amino acid, blood, pooling, protein

INTRODUCTION

The ability to influence production parameters, specifically milk protein, is of considerable interest to dairy farmers and nutritionists. Milk protein production is heavily influenced by how efficiently cows use MP for milk protein synthesis and by essential AA profiles found in this MP. There has been substantial research indicating that, according to the NASEM (2001) model, Met is a limiting AA for dairy cows fed corn-silage and alfalfa-silage diets common in the United States (NASEM, 2001; Schwab and Broderick, 2017). Rumen-protected Met (**RPM**) was developed to supply Met in a form protected from rumen degradation and available for absorption across the small intestine. Both postruminal Met infusion and dietary rumen-protected Met supplementation increase concentration and yield of milk protein and milk fat (Zanton et al., 2014). Supplementing RPM to improve EAA profiles could also allow for the reduction in RUP of diets, reducing the amount of dietary N and subsequent environmental losses (NASEM, 2001).

Bioavailability of rumen-protected AA products can vary widely, depending on encapsulation technique and chemistry, but relative differences can be ascertained though analyzing plasma free AA response to product supplementation (Rulquin and Kowalczyk, 2003; Whitehouse et al., 2017). Free plasma AA is a strong indicator of bioavailability to animals and responds linearly to intestinal absorption (Rulquin and Kowalczyk, 2003). When cows are fed isonitrogenous diets, differences in availability of RPM products can be assessed by evaluating differences in plasma free Met (Papas et al., 1984; Overton et al., 1996; Südekum et al., 2004). The primary objective of this study was to compare the plasma Met levels of cows fed a newly developed RPM product with those of cows fed 2 existing products with known differences in plasma Met response. We hypothesized that the new product would elicit comparable plasma Met responses to that of the more available product, due to similarities in protective technology. Also, because measurement of free AA in plasma samples is costly, a secondary objective was

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to determine how pooling of individual cow samples may have affected data interpretation.

MATERIALS AND METHODS

Animals and Treatments

This study was approved by the University of Delaware Institutional Animal Care and Use Committee and was conducted from November to December of 2018.

Ten multiparous Holstein cows in mid to late lactation were moved to a tie-stall facility 7 d before the start of the experiment for a 1-wk adaptation period. Cows used had a mean DIM of 280 (±73) at the beginning of the trial. Cows were fed 3 times daily at approximately 0800, 1630, and 2400 h, with 33% of their daily feed allotment provided at each feeding. Cows were fed ad-libitum for ~5% orts. Cows had free access to individual waterers throughout the study. Cows were milked twice daily (~0430 and 1530 h). The average BW of the 10 cows measured on 2 consecutive days at the end of the adaptation period was 755 (±56) kg.

The experiment was conducted over 4 wk, with the 1-wk adaptation period followed by three 7-d periods using a replicated 3 × 3 Latin square design. During the adaptation period, cows were fed a diet that had been formulated using CNCPS v6.5 (Van Amburgh et al., 2015) to contain sufficient levels of all essential AA (Table 1). At the end of the adaptation period cows were assigned to blocks by DIM and randomly assigned to treatment sequences within each block. Nine cows were assigned to 3 complete blocks, and the remaining cow was assigned to an incomplete block.

The 3 experimental treatments consisted of the control diet plus 12 g/d of either KESSENT M (**RPM-K**; Kemin Industries), Smartamine M (**RPM-S**; Adisseo Inc.), or Mepron (**RPM-M**; Evonik Nutrition & Care GmbH). The RPM-S treatment was used as a positive control because it has a high Met bioavailability of approximately 80% (Rulquin and Kowalczyk, 2003). The RPM-M treatment was included as a negative control because it has a lower bioavailability when assessed by comparing the plasma free Met response to RPM-S (Blum et al., 1999; Südekum et al., 2004). Before each feeding time, ~1 kg of TMR was mixed into small tubs with treatments and placed in front of the cows. Cows were given access to the TMR and supplements for 15 min. If the mix was not consumed within 15 min, the remainder was swept up and placed on top of fresh feed. The RPM-K was provided by Kemin Industries Inc., and RPM-S and RPM-M were purchased from Renaissance Nutrition.

Table 1. Ingredient composition and analyzed nutrient content of the experimental diet

Item	Value
Ingredient, % DM	
Corn silage	38.60
Alfalfa silage	13.74
Ground corn grain	17.22
Canola meal	15.23
Expelled soybean meal	5.50
Corn gluten meal	5.35
Calcium carbonate	0.91
Sodium bicarbonate	0.76
Rumen bypass fat[1]	0.67
Trace mineral and vitamin mix[2]	0.49
Potassium carbonate[3]	0.48
Sodium chloride	0.43
Sugar by-product[4]	0.32
Vegetable fat[5]	0.29
Biotin[6]	0.004
Live yeast[7]	0.002
DM, %, ± SD	49.5 ± 1.2
Nutrient, % DM ± SD	
CP[8]	20.9 ± 0.1
aNDF[9]	28.3 ± 0.6
ADF	18.0 ± 0.6
Starch	22.6 ± 0.8
Ash	8.4 ± 0.6
NE_L[10]	1.66 ± 0.01

[1]MEGALAC (Church & Dwight Co. Inc.).

[2]Contained 5.8% calcium, 34.4% magnesium, 7.3% sulfur, 4.5% potassium, 52 mg/kg Fe, 7,093 mg/kg Zn, 1,223 mg/kg Cu, 5,303 mg/kg Mn, 65 mg/kg Se, 141 mg/kg Co, 191 mg/kg I, 882 KIU/kg vitamin A, 220 KIU/kg vitamin D, and 5,292 IU/kg vitamin E.

[3]DCAD Plus (Church & Dwight Co. Inc.).

[4]Contained 92.3% sucrose.

[5]Palmit 80 (Global Agri-trade Corporation).

[6]Microvit H Promit Biotin 2% (Addiseo).

[7]Levucell SC (Lallemand Animal Nutrition).

[8]Using CNCPS v6.5 (Van Amburgh et al., 2015) and average DMI, milk production, and milk composition collected during the experiment, predicted grams per day required and supplied were 92.1 and 202.8 for Arg, 76.1 and 89.4 for His, 120.3 and 171.9 for Ile, 198.8 and 324.1 for Leu, 187.1 and 207.8 for Lys, 70.5 and 80.6 for Met, 122.5 and 177.8 for Phe, 105.6 and 163.7 for Thr, 29.6 and 44.6 for Trp, and 132 and 193.9 for Val.

[9]aNDF = NDF assayed with a heat-stable amylase and sodium sulfite and expressed inclusive of residual ash.

[10]Calculated using NASEM (2001).

Milk and Feed Sampling

Milk yield was recorded for each cow at each milking throughout the study, and milk samples were collected during morning and afternoon milkings (~0430 and 1530 h) d 5 to 7 of each period and submitted to Dairy One for near-infrared analysis of lactose, protein, fat, SCC, and MUN using a MilkoScan FT+ (Foss).

Feed offered and refused was recorded daily. Samples of wet forages were collected 3 times a week during the

morning feeding and composited by week. The grain mix was sampled once weekly. A portion of each feed sample was dried for 48 h at 60°C in a forced-air oven, and results were used to correct the diet for DM fluctuation. Weekly composite samples were mailed to Cumberland Valley Analytical Services for wet chemistry analysis of DM (105°C for 3 h for forages; method 930.15, AOAC International, 2000, for grain), **aNDF** (NDF assayed with a heat-stable amylase and sodium sulfite and expressed inclusive of residual ash; Van Soest et al., 1991), ADF (method 973.18, AOAC International, 2000), CP (method 990.03, AOAC International, 2000), starch (Hall, 2009), and ash (method 942.05, AOAC International, 2000).

Blood Sampling

Blood samples were collected 2 and 6 h after feeding on the last day of the adaptation period from a coccygeal vessel. During each experimental period, jugular catheters (ICU Medical Inc.) were placed in each cow on d 4. Blood samples were collected on d 5 to 7 of each period at 2, 4, 6, and 8 h after the first feeding. A total of 10 mL of blood was collected at each time point into EDTA-coated tubes (Becton Dickinson). Blood samples were centrifuged at 2,000 × *g* for 20 min at 4°C after each collection, and isolated plasma was stored in cryovials at −80°C. Jugular catheters were removed following the last blood sample taken on d 7 of each period. At the end of the experiment, plasma samples were mailed to the University of Missouri Agriculture Experiment Station Chemical Laboratories for AA analysis. Analysis was conducted on a L-8900 Amino Acid Analyzer (Hitachi) following the procedure of Le Boucher et al. (1997). Plasma samples were deproteinized with 40 g/L sulfosalicylic acid before analysis (Le Boucher et al., 1997). Specifically, a 40% solution of sulfosalicylic acid was prepared and added to plasma in a 1:10 ratio.

Statistical Analysis

For DMI and milk yield, mean data from the last 3 d of each experimental period were used. Mean milk component data from the last 3 d of each period were weighted by milk yield at each individual milking. Data were evaluated using the GLIMMIX procedure of SAS (SAS Institute Inc.) using a model that included the fixed effects of treatment, period, and block and the random effect of cow.

Plasma free AA concentrations were evaluated using the GLIMMIX procedure of SAS using a model that included fixed effects of treatment, period, block, day of sampling, and hour of sampling, and all 2- and 3-way interactions of treatment, day, and hour. Cow was included as a random effect, and mean plasma concentration of that AA from the adaptation period was included as a covariate. Hour was included as a repeated measure with the subject of period × day × cow, and a first-order autoregressive covariance structure was used.

To assess whether similar results would have been obtained had samples been pooled by day or by period, mean plasma Met concentrations were determined for each cow within a day (mean of the 2-, 4-, 6-, and 8-h samples) and for each cow within a period (mean of the 12 samples collected over the 3 d). Daily means were evaluated using the same model used for the full data set except that hour and interactions with hour were excluded from the model and the repeated measure subject was period × day. The model was further refined to evaluate the period means by removing day, interactions with day, and the repeated statement from the model.

For all models, significance was declared at $P \leq 0.05$, and trends were discussed at $0.05 < P \leq 0.10$. When a fixed effect was observed ($P \leq 0.10$), the pdiff function of SAS was used to differentiate among effect levels.

RESULTS AND DISCUSSION

Ingredient and nutrient compositions of the basal diet are in Table 1. The diet was formulated to contain 20.5% CP, 29.7% aNDF, 17.9% ADF, 22.0% starch, 7.6% ash, and 1.68 Mcal/kg NE_L. The analyzed nutrient composition of the diet (Table 1) was similar to formulated values. Using CNCPS v6.5 (Van Amburgh et al., 2015) and average DMI, milk production, and milk composition collected during the experiment, Met and Lys were predicted to be 2.24 and 5.76% of MP, respectively. Predicted required and supplied amounts of Met were 70.5 and 80.6 g/d, respectively, and required and suppled Lys was 187.1 and 207.8 g/d, respectively. All other EAA were similarly predicted to be supplied at levels greater than requirements (predicted g/d required and supplied were 92.1 and 202.8 for Arg, 76.1 and 89.4 for His, 120.3 and 171.9 for Ile, 198.8 and 324.1 for Leu, 122.5 and 177.8 for Phe, 105.6 and 163.7 for Thr, 29.6 and 44.6 for Trp, and 132 and 193.9 for Val).

Production Response

There were no effects of treatment on DMI, milk yield, or milk composition (Table 2). Milk urea nitrogen was elevated at approximately 17 mg/dL, and milk protein was high at approximately 3.65% across all treatments, suggesting that we achieved our goal of exceeding dietary AA requirements. The lack of treatment differences in milk production or composition was as expected, as diets were formulated to exceed Met requirements. Thus, any additional MP Met supply from more available RPM sources was not expected to overcome a deficiency and enhance production. This result was desirable as it suggests that supplemented RPM products should be detectable in the blood stream versus diverted for productive use.

Plasma AA

Plasma Met was affected by treatment ($P < 0.001$; Table 3) and hour ($P = 0.001$; Table 4). Interactions of treatment × hour, treatment × day, hour × day, and treatment × hour × day were not found ($P > 0.16$). The absence of

Table 2. Effect of treatment on DMI, milk production, and milk composition

	Treatment[1]				*P*-value
Item	RPM-S	RPM-K	RPM-M	SEM	Treatment
DMI, kg/d	27.6	27.8	27.5	1.0	0.82
Milk, kg/d	31.5	32.4	30.8	3.9	0.13
Fat, %	4.07	4.02	4.18	0.19	0.29
Fat, kg/d	1.24	1.26	1.26	0.12	0.93
Protein, %	3.66	3.63	3.69	0.06	0.20
Protein, kg/d	1.15	1.17	1.13	0.14	0.27
Lactose, %	4.51	4.49	4.49	0.04	0.80
MUN, mg/dL	16.6	17.0	17.2	0.7	0.43
SCS[2]	4.09	4.23	4.12	0.73	0.93

[1]Cows were provided a control diet plus 12 g/d of KESSENT M1 (RPM-K; Kemin Industries Inc.), Smartamine M2 (RPM-S; Adisseo USA Inc.), or Mepron3 (RPM-M; Evonik Nutrition & Care).

[2]SCS = somatic cell score = $\log_2$(SCC/100,000) + 3.

a day effect ($P = 0.54$) indicated that the plasma response had stabilized by the first day of blood sampling on d 5 of each period. There were no differences between RPM-S and RPM-K (32.7 vs. 33.0 μM; $P = 0.75$), and both were greater than RPM-M (30.1 μM; $P < 0.001$). The time effect was due to plasma Met being lowest at 4 h as compared with levels at 2, 6, and 8 h after feeding (Table 4). A decrease at 4 h has sometimes been observed in other studies with a similar design (N. L. Whitehouse, University of New Hampshire, Durham, NH, personal communication).

The interaction of treatment × hour did not affect any AA except for a tendency that was observed for Phe ($P = 0.08$). This was due to a lower concentration of Phe for RPM-K at 4 h (58.4 μM) compared with RPM-K at 2 h, RPM-M at 4 h, and RPM-S at 6 h (63.6, 64.0, and 64.0 μM, respectively, $P < 0.05$, data not shown). In addition to Met, treatment also affected cystine ($P = 0.009$) and tended to affect Ala ($P = 0.06$), Leu ($P = 0.07$), and Val ($P = 0.053$). Cystine followed the same pattern as Met, with no difference between RPM-S and RPM-K ($P = 0.42$), but both were greater than RPM-M ($P \leq 0.03$). The tendencies for effect of treatment on Ala, Leu, and Val were due to Ala being greater in RPM-K than RPM-S ($P = 0.02$), Leu being greater in RPM-M than RPM-S ($P = 0.02$), and Val being lower in RPM-S than RPM-M ($P = 0.02$) or RPM-K ($P = 0.09$).

An effect of hour of sampling occurred or tended to occur for all plasma AA except for Phe and Val (Table 4). In general, greater concentrations were observed at 6 or 8 h and lower concentrations were observed at 2 or 4 h. Time effects for Arg and Asn were the same as observed for Met, with lower plasma levels at 4 h compared with all other times. For Leu, Lys, Thr, and Tyr, plasma concentrations at 4 h were less than those at 6 and 8 h, but that at 2 h did not differ from any other time. For Ala and cystine, plasma concentrations at 2 and 4 h were both lower than 6 and 8 h. For Asp, lower values were observed at 2 h than at 6 or 8 h, and 4 h did not differ from any other time. For Gln and Pro, concentrations at 4 h were lower than at 6 h, and 6 h was lower than both 2 and 8 h, which did not differ from one another. For Gly and His, plasma concentrations were lowest at 4 h and greatest at 8 h. Intermediate levels were found at 2 and 6 h, which differed from both 4 and 8 h. For Ile, 6 h was greater than 2 or 4 h, and 8 h did not differ from any other time. For Ser, 4 h was lower than all other times, 2 h was lower than 8 h, and 6 h did not differ from 2 or 8 h. For Trp, there was no difference between 2 and 4 h, which were both lower than 6 h, and 8 h was greater than all other times. A difference in this pattern was noted for Glu, where plasma concentrations at 4 h were greater than those at 2 or 8 h, and 6 h was also greater than 8 h.

The RPM-S and RPM-M treatments were included as positive and negative controls, respectively, due to their demonstrated differences in plasma Met response (Blum et al., 1999; Südekum et al., 2004). Differences in plasma Met concentrations between RPM-M and RPM-S most likely reflect differences in degrees of protection of Met against ruminal degradation or availability for intestinal absorption. In an in vitro study using rumen inoculum from sheep, Mbanzamihigo et al. (1997) demonstrated that RPM-M had reduced protection from rumen degradation than RPM-S. Using the mobile bag technique, small intestinal disappearance of RPM-M was 44% (Berthiaume et al., 2000), and total postruminal disappearance was to 63 to 78% (Overton et al., 1996; Berthiaume et al., 2000) for bags that had been preincubated in the rumen for 4.5 to 6 h. These results suggest that intestinal availability may be lower for RPM-M than RPM-S, though the mobile bag technique may underestimate availability (Berthiaume et al., 2000). Lower rumen stability or reduced

Table 3. Plasma free AA concentrations (μ*M*) for cows provided a control diet plus 12 g/d of KESSENT M[1] (RPM-K), Smartamine M[2] (RPM-S), or Mepron[3] (RPM-M)

	Treatment				*P*-value	
AA	RPM-S	RPM-K	RPM-M	SEM	Treatment	Treatment × hour[4]
Alanine	236	249	242	5	0.06	0.38
Arginine	78.6	79.6	77.8	1.7	0.67	0.20
Asparagine	55.9	57.4	56.7	1.9	0.64	0.26
Aspartate	4.07	4.11	4.15	0.19	0.89	0.55
Cystine	18.3[A]	18.5[A]	17.5[B]	0.5	0.009	0.76
Glutamate	39.3	38.1	38.4	1.1	0.18	0.40
Glutamine	295	299	298	7	0.78	0.53
Glycine	239	245	238	5	0.52	0.66
Histidine	69.1	70.2	70.2	1.8	0.50	0.18
Isoleucine	167	170	173	7	0.30	0.22
Leucine	327	333	343	17	0.07	0.15
Lysine	87.7	90.0	88.6	2.7	0.67	0.26
Methionine	32.7[A]	33.0[A]	30.1[B]	0.8	0.001	0.16
Phenylalanine	61.6	61.6	63.3	1.5	0.83	0.08
Proline	110	115	113	4	0.37	0.39
Serine	91.5	96.1	94.0	2.5	0.11	0.38
Threonine	104	107	103	4	0.27	0.48
Tryptophan	38.5	38.5	39.6	1.0	0.33	0.52
Tyrosine	67.5	67.8	70.7	3.1	0.17	0.16
Valine	403	416	421	13	0.053	0.24

[A,B]Within a row, means with unlike superscripts differ ($P \leq 0.05$).

[1]KESSENT M (Kemin Industries Inc.).

[2]Smartamine M (Adisseo USA Inc.).

[3]Evonik Nutrition & Care.

[4]A total of 4 g of 1 of 3 rumen-protected Met products was dosed every 8 h (12 g/d). Plasma samples were collected 2, 4, 6, and 8 h after the first dosing time.

intestinal availability of RPM-M compared with RPM-S may explain the lower plasma response observed with that treatment.

Our results were consistent with studies that demonstrated that changes in plasma AA concentrations can be used to differentiate availability of different rumen-protected AA products (Ordway et al., 2009; Whitehouse et al., 2017). Südekum et al. (2004) reported increases in plasma Met in steers fed a single daily pulse dose of 50 g of Met from RPM-M and RPM-S; however, only RPM-S significantly increased plasma Met above basal plasma values (463.6 vs. 23.0 μ*M*, respectively), whereas the increase for RPM-M was not significantly above baseline (38.8 vs. 24.5 μ*M*). Similarly, Blum et al. (1999) reported that a single daily dose of 50 g of Met from RPM-S fed to lactating dairy cows caused a 9-fold increase in free plasma Met compared with baseline (144.8 vs. 16.6 μ*M*, respectively), whereas a 2-fold increase was observed when feeding 50 g of Met from RPM-M (29.3 vs. 15.7 μ*M*, respectively). Although our study was not designed to evaluate changes from baseline, plasma samples collected during the adaptation period had a mean plasma free Met concentration of 25.7 μ*M*. Thus, supplementation with RPM-S and RPM-M resulted in numeric increases of 27 and 17%, respectively, relative to the adaptation period. Due to the lower level of supplementation in the present experiment (9.0 g/d DL-Met for RPM-S and 10.2 g/d for RPM-M split into 3 daily feedings), the magnitude of the response was less than that observed by Blum et al. (1999) and Südekum et al. (2004) but resulted in similar ranking of the products. These results support their use as positive and negative controls in the present experiment, respectively.

The RPM-M product contains a core of 85% DL-Met protected by thin coats of stearic acid and ethylcellulose that allow for slow release (Schwab, 1995). The approximately 75% DL-Met core of RPM-S is protected by ethylcellulose covered with stearic acid containing poly(2-vinylpyridine-co-styrene), allowing for ruminal protection and pH dependent release (Schwab, 1995). The more recently developed RPM-K product, like RPM-S, uses a pH sensitive copolymer coating around a DL-Met core. Specifically, a vinylpyridine/styrene copolymer resists degradation in

Table 4. Effect of time of sampling on plasma free AA concentrations (μ*M*) for cows provided a control diet plus 12 g/d of KESSENT M,[1] Smartamine M,[2] or Mepron[3]

	Hour					*P*-value
AA	2	4	6	8	SEM	Hour[4]
Alanine	238^{B}	234^{B}	246^{A}	251^{A}	8	0.001
Arginine	79.8^{A}	74.2^{B}	79.3^{A}	81.3^{A}	1.8	0.004
Asparagine	56.5^{A}	52.8^{B}	58.2^{A}	59.2^{A}	1.9	0.001
Aspartate	3.79^{B}	4.05^{AB}	4.28^{A}	4.33^{A}	0.2	0.008
Cystine	17.2^{B}	17.5^{B}	18.7^{A}	18.9^{A}	0.5	0.001
Glutamate	38.2^{BC}	39.6^{A}	39.3^{AB}	37.4^{C}	1.1	0.001
Glutamine	303^{A}	281^{C}	294^{B}	311^{A}	7	0.001
Glycine	237^{B}	223^{C}	243^{B}	260^{A}	4	0.001
Histidine	69.5^{B}	68.2^{C}	70.0^{B}	71.7^{A}	1.7	0.001
Isoleucine	165^{B}	168^{B}	175^{A}	171^{AB}	7	0.02
Leucine	330^{AB}	327^{B}	340^{A}	340^{A}	17	0.03
Lysine	88.2^{AB}	83.7^{B}	90.3^{A}	92.8^{A}	2.8	0.009
Methionine	32.6^{A}	30.2^{B}	31.9^{A}	33.0^{A}	0.8	0.001
Phenylalanine	62.0	61.0	62.8	61.6	1.5	0.42
Proline	116^{A}	106^{C}	111^{B}	117^{A}	4	0.001
Serine	93.3^{B}	88.8^{C}	95.6^{AB}	97.7^{A}	2.4	0.001
Threonine	105	101	106	107	4	0.07
Tryptophan	36.6^{C}	35.7^{C}	40.4^{B}	42.8^{A}	1.0	0.001
Tyrosine	69.3	66.2	69.5	69.7	3.1	0.10
Valine	412	407	417	417	13	0.12

$^{A–C}$Within a row, means with unlike superscripts differ ($P \leq 0.05$).

[1]KESSENT M (Kemin Industries Inc.).

[2]Smartamine M (Adisseo USA Inc.).

[3]Evonik Nutrition & Care.

[4]A total of 4 g of 1 of 3 rumen-protected Met products was dosed every 8 h (12 g/d). Plasma samples were collected 2, 4, 6, and 8 h after the first dosing time.

the rumen and begins releasing Met at the acidic environment found in the abomasum. Differences among RPM-S, RPM-K, and RPM-M in plasma Met response may be due to differences in protective technology, with the former 2 using a pH-sensitive coating and the latter using time-dependent release. Räisänen et al. (2020) observed a greater increase in plasma Met levels in lactating dairy cows given pH-sensitive polymer treatment as compared with lipid- or ethyl cellulose–protected treatments.

An effect of time on plasma Met is not unexpected as the RPM products were pulse dosed every 8 h, and differences over time may be related to kinetics of passage and absorption of the products. However, similar time effects were observed for other plasma AA, suggesting this effect may have been driven by timing of TMR allocation. Effect of meal pattern on timing of plasma AA appearance has been demonstrated in pigs. When examining the arterial concentrations of AA in pigs fed various diets in equal amounts 3 times daily, Reverter et al. (2000) reported increases in most AA following feeding, with the timing of peak ranging from 30 to 120 min. Similarly, Agyekum et al. (2016) observed peak arterial concentrations of most AA at 180 min following feeding in pigs fed once per day. However, similar responses to time are less likely to be observed in ruminants because more consistent rumen emptying reduces fluctuation in digesta flow to the duodenum (Whitt et al., 1996). A recent study using lactating cows fed once daily a diet supplemented with RPM-M found no effect of time following feeding on plasma Met, though temporal effects were observed for Lys and tended to occur for His (Toledo et al., 2021). Although ruminal retention affects the time frame between feed consumed and appearance of AA in the blood stream when comparing monogastrics to ruminants, our data support a temporal response to feeding on the appearance of AA in the blood similar to that observed in monogastrics.

Though our data suggest that the time effect on plasma Met was most likely driven by the time of feeding versus the time of product dosing, multiple previous studies have indicated a relationship between time of RPM treatments and plasma Met appearance. Bach and Stern (2000) administered a single pulse dose of different RPM products using an esophageal bolus gun. Plasma Met peaked by 12 h for more slowly degradable products and between 6 and

12 h for moderately degradable RPM. Similarly, Koenig and Rode (2001) found that plasma Met concentration peaked 9 to 12 h following a single oral dose of RPM-M. In cows fed RPM-S, Graulet et al. (2005) observed peak plasma Met at 22 h following a single intraruminal dose, and Toledo et al. (2017) found peak plasma Met at 12 h when cows were fed RPM-S once daily. In the present study, treatments were administered every 8 h, and 12 h following product dosing (the most common time that others observed peak response to a pulse dose of RPM) equates to our 4-h sampling point. Because we observed the lowest plasma Met concentration at 4 h, this again suggests that the temporal effect was due to timing of TMR allocation rather than timing of Met dosing.

Effect of Plasma Sample Pooling

Analyzing plasma AA content is often one of the most expensive components of experiments evaluating plasma AA response to feeding RPM products. For this experiment we analyzed a relatively large number of plasma samples for each cow during each period (4 per day for each of 3 d), allowing us to retrospectively evaluate how pooling of samples might have affected results. When using the plasma free AA response to estimate bioavailability, Whitehouse et al. (2017) recommended pooling multiple samples collected during a day into a single daily aggregate. We used this approach with our data set by calculating individual daily means for each cow and analyzing those in a separate model. We then took this one step further by calculating individual period means for each cow. For the full model, daily mean model, and period mean model, the *P*-value for the effect of treatment on plasma free Met was 0.001, 0.04, and 0.07, respectively, and SEM was 0.75, 0.87, and 0.94, respectively. Relative differences between treatments were maintained in all 3 models; however, for the period mean model, RPM-S only tended to differ from RPM-M ($P = 0.06$). These results support pooling samples by day to save on analytical costs, but pooling by period would have required additional animals to maintain significant differences. The strong effect of time of sampling on plasma AA (Table 3) confirms the recommendation of Whitehouse et al. (2017) to collect multiple samples each day.

APPLICATIONS

Plasma free Met was effective at ranking a new product relative to 2 products with known differences in bioavailability. Both RPM-K and RPM-S displayed greater plasma Met than the RPM-M product, suggesting that the bioavailability of both RPM-K and RPM-S are comparable and greater than that of RPM-M. There was an effect of time of sampling on plasma Met and most of the other AA, demonstrating the importance of collecting multiple samples after feeding when assessing plasma AA response. Pooling individual cow plasma samples by day would have likely resulted in the same differentiation of treatments, but pooling by period would have masked some of these differences.

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