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Evaluation of animal performance responses to the supplementation of 2 rumen-protected methionine supplements in post-peak-lactation Holstein cows

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ABSTRACT

Objective: Our objective was to evaluate the performance response of dairy cows fed a Met-deficient diet and provided a rumen-protected Met (RPM) supplement.

Materials and Methods: A total of 24 multiparous (95 ± 20 DIM) and 6 primiparous (71 ± 3 DIM) Holstein cows were assigned to 1 of 3 treatments using a replicated 3 × 3 Latin square design with 21-d periods: a control diet deficient in MP Met by 17 g/d (CON) or the control diet plus 14 g/d of a RPM supplement, RPM-K or RPM-S, containing approximately 80% Met. Milk samples were collected on d 13 to 14 and 18 to 21 of each period. Plasma samples were collected on d 21 of each period. Contrasts were used to evaluate the effect of RPM addition (CON vs. RPM-K + RPM-S combined) and source of RPM (RPM-K vs. RPM-S).

Results and Discussion: There was no difference between RPM-S and RPM-K in milk fat percentage (3.66 vs. 3.69%, respectively; $P = 0.47$) or milk protein percentage (3.28% for both treatments; $P = 0.98$), but RPM-K decreased DMI compared with RPM-S (26.2 vs. 26.6 kg/d; $P = 0.02$). Milk fat and protein percentages were increased by RPM relative to CON (3.60% fat and 3.25% protein; $P = 0.02$ and $P = 0.04$, respectively). Milk fat yield was not different between RPM-K and RPM-S (1.39 and 1.40 kg/d; $P = 0.74$), but milk fat yield tended to increase with RPM relative to CON (1.37 kg/d, $P = 0.07$). Plasma free Met was not different between RPM-S and RPM-K treatments (46.6 and 46.5 μM, respectively; $P = 0.97$), and RPM supplementation increased plasma concentrations relative to CON (33.0 μM; $P < 0.001$).

Implications and Applications: Relative to CON, both RPM supplements similarly increased milk fat, milk protein, and plasma free Met, suggesting similar relative bioavailability.

Key words: milk protein, milk fat, plasma methionine

INTRODUCTION

Dairy cows have relatively high requirements for absorbed EAA to support milk and milk component yields. Milk protein yield is responsive to increased supply of limiting AA including Met, Lys, Ile, and His (NASEM, 2021). The response to supplemental Met has been most extensively studied, and rumen-protected Met (**RPM**) supplementation has been observed to increase milk protein percentage and yield (Zanton et al., 2014; Toledo et al., 2021). Additional benefits of increased Met in the diet may include increased milk yield, milk fat content, and ECM (Osorio et al., 2013; Zanton et al., 2014) as well as improved immune function and health of periparturient cows (Coleman et al., 2021). Other benefits of supplementing rumen-protected AA are the potential for reducing dietary protein and subsequent environmental N losses (Dinn et al., 1998; NASEM, 2001).

The objective of this experiment was to evaluate production response of post-peak-lactation dairy cows to 2 supplements (**RPM-K** and **RPM-S**) designed to protect Met from rumen degradation. Both supplements protect Met with a pH-sensitive polymer that is resistant to ruminal degradation and releases in the intestines. Due to the similarity in technology, we hypothesized that RPM-K and RPM-S would similarly increase milk component yields and plasma Met concentrations relative to an unsupplemented control treatment.

MATERIALS AND METHODS

This study was approved by the University of Delaware Animal Care and Use Committee and was conducted from October 2019 through January 2020.

Animals and Treatments

The experiment used 30 Holstein cows, 24 multiparous and 6 primiparous, with a mean DIM at the start of period 1 of 95 (±20) and 71 (±3), respectively. Cows were moved to a barn with a Calan Broadbent Feeding System

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(American Calan Inc.) and trained over a 2-wk period before the start of the experiment. After the training period, cows were transitioned to the control (**CON**) diet (Table 1) for a 14-d adaptation period. The CON diet was formulated using CNCPS v6.55 (Van Amburgh et al., 2015) to be deficient in Met by approximately 17 g/d based on a target of 1.13 g of Met/Mcal of ME. The Lys supply was sufficient to achieve the target of 3.05 g of Lys/Mcal of ME. The diet was formulated to provide 2,670 g/d MP with 62.8 and 216.6 g/d absorbed Met and Lys, respectively. Following completion of the study, NASEM (2021) was used with mean animal inputs and feed nutrient composition to predict AA flows. Target supply and predicted supply were 61 and 50 g/d, respectively, for Met and 192 and 209 g/d, respectively, for Lys. Predicted MP supply was 2,252 g/d, and predicted requirement was 2,536 g/d.

At the end of the adaptation period, cows were blocked by parity, DIM, and milk yield and assigned to replicated 3 × 3 Latin squares. Each treatment period lasted 3 wk. The 3 treatments consisted of the CON diet or the CON diet plus 14 g/d of either KESSENT M (RPM-K; Kemin Industries Inc.) or Smartamine M (RPM-S; Adisseo Inc.). The RPM-K treatment was provided by Kemin Industries Inc., and RPM-S was purchased from Renaissance Nutrition. According to manufactures specifications, both RPM-S and RPM-K contain approximately 75% DL-Met; thus, 14 g/d of supplement contained 10.5 g of Met. Body weight was measured on 3 consecutive days at the end of each experimental period.

During the adaptation and experimental periods, cows were fed *ad libitum* once daily (~0800 h), allowing for 5 to 10% refusals. During the experimental periods, supplements (RPM-K or RPM-S) were top dressed in equal amounts twice daily, at the time of feeding (~0800 h) and before return from the afternoon milking (~1530 h).

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

Item	Value
Ingredient, % DM	
Corn silage	47.92
Triticale silage	8.96
Ground corn grain	15.65
Soybean meal	7.35
Canola meal	7.29
Dehydrated citrus pulp	7.17
Rumen bypass fat[1]	1.38
Sugar by-product[2]	1.02
Sodium bicarbonate	0.82
Calcium carbonate	0.50
Mineral and vitamin mix[3]	0.46
Urea	0.45
Sodium chloride	0.39
Rumen-protected lysine[4]	0.27
Monocalcium phosphate	0.21
Potassium magnesium sulfate	0.18
DM, %	48.3
Nutrient, % DM ± SD	
CP	15.4 ± 0.3
aNDF[5]	29.2 ± 1.7
ADF	18.3 ± 0.6
Starch	27.5 ± 1.3
Ash	7.0 ± 0.5

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

[2]Contained 92.3% sucrose (Renaissance Nutrition Inc.).

[3]Contained 14.8% Ca, 34.0% Mg, 0.8% S, 105 mg/kg Fe, 4,213 mg/kg Zn, 817 mg/kg Cu, 4,200 mg/kg Mn, 65.1 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.

[4]LysiGEM (Kemin Industries).

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

Milk and Feed Sampling

Cows were milked twice daily (~0430 and ~1530 h). Milk yield was recorded at each milking throughout the study. During each experimental period, milk samples were collected on d 6 and 7 of wk 2 and d 4 through 7 of wk 3. Milk samples were submitted to Dairy One for analysis of lactose, protein, fat, SCC, and MUN using a MilkoScan FT+ (Foss).

Feed offered and refused was recorded daily. Samples of wet forages and TMR were collected 3 times a week and composited by week, and the grain mix was sampled once per week. A portion of each forage and grain sample was dried for 48 h at 60°C in a forced-air oven, and results were used to adjust feed mixing amounts to account for DM changes. Weekly composite samples were analyzed using wet chemistry methods by Cumberland Valley Analytical Services. Samples were analyzed for 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, with results 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). Samples of RPM-S and RPM-K were also mailed to Cumberland Valley Analytical Services for analysis of CP and DM.

Blood Sampling

Plasma samples were collected at 2 and 6 h after feeding on the last day of each experimental period. A total of 10 mL of blood was collected at each time point into EDTA-coated tubes (Becton Dickinson). Blood was centrifuged at 2,000 × *g* for 20 min at 4°C after each collection, and plasma was stored at −80°C until it was mailed to the University of Missouri Agriculture Experiment Station Chemical Laboratories for AA analysis. Amino acid

analysis followed the procedure of Le Boucher et al. (1997) and was conducted using a L-8900 Amino Acid Analyzer (Hitachi). Samples were deproteinized with 40 g/L sulfosalicylic acid (a 40% solution of sulfosalicylic acid in water was added to plasma in a 1:10 ratio) before analysis.

Statistical Analysis

Statistical Models. For wk 2 and 3 of each period, weekly means of intake and milk yield were calculated. Weighted means of milk composition data were also determined (weighted means of 4 samples collected over d 6–7 for wk 2 and weighted means of 8 samples collected over d 4–7 for wk 3). Milk production, milk composition, and intake data were evaluated using the MIXED procedure of SAS (SAS Institute Inc.) using a model containing fixed effects of treatment, period, week, parity, block, and the interaction of treatment by week and the random effect of cow. Week was included as a repeated measure with the subject of cow × treatment, and a first-order autoregressive covariance structure was used. Responses to treatment were determined using the CONTRAST statement of SAS and 2 nonorthogonal contrasts. Estimates were not adjusted for nonorthogonality. The RPM contrast compared CON with RPM supplementation (the RPM-S and RPM-K treatments combined), and the source contrast compared RPM-S with RPM-K.

Milk samples were collected during both wk 2 and 3 of each period. This was done because we expected milk component responses to RPM supplementation to plateau by 2 wk following supplementation. Data were initially analyzed using 2 models, 1 containing only the wk-3 data and 1 containing the combined data from both wk 2 and 3 as described previously. Predicted LSM were similar for both models. However, errors were reduced with the larger data set, so results from both weeks were included in the final model.

Body weight was evaluated using the same model except that week and the interaction of treatment by week were removed from the model. Plasma samples were collected twice each period, on d 7 of wk 3 at 2 and 6 h following feeding. Plasma AA data were evaluated using the same model used to evaluate milk and intake data, except that the repeated factor of week used for the milk and intake data was replaced by hour of blood sampling.

The interaction of treatment by parity was initially included in all models but was never significant ($P > 0.25$) and was removed from the final models. For all models, significance was declared at $P \leq 0.05$, and trends were discussed at $0.05 < P \leq 0.10$.

Removal of Outliers. One primiparous cow developed clinical mastitis at the start of the second period and was excluded from analysis. Three additional cows (2 multiparous and 1 primiparous) were excluded from analysis due high variability in milk yield and DMI unrelated to treatment. Residuals from the full models for milk yield and DMI were tested for outliers using the VBOX option of the SGPLOT procedure of SAS. The 3 cows that were excluded had multiple data points identified as outliers in each model.

RESULTS AND DISCUSSION

Diet

The diet was formulated using CNCPS v6.5 to contain 15.0% CP, 30.4% aNDF, 18.0% ADF, 25.5% starch, and 6.8% ash. Analyzed nutrient composition of the TMR was similar to formulated values except for starch, which analyzed 2 percentage units greater than formulated levels (Table 1). Formulated NE_L and ME supply were 1.79 Mcal/kg and 70.5 Mcal/d, respectively.

Production Response

The contrast for the effect of source on DMI was significant ($P = 0.02$), with lower DMI for cows on the RPM-K treatment than for those on RPM-S (26.2 vs. 26.6 kg/d; Table 2). This effect was unexpected, as supplementation with different RPM sources does not typically affect DMI (Zang et al., 2017; Ardalan et al., 2021). However, in their meta-analysis, Zanton et al. (2014) found that supplementation with RPM-S increased DMI by 0.31 kg/d relative to control cows, and supplementation with Mepron (Evonik Industries) decreased DMI by 0.25 kg/d relative to control cows. There has been little published work on RPM-K, and this effect warrants further evaluation.

Treatment did not affect yields of milk, milk protein, ECM, ECM divided by DMI, somatic cell score, MUN, or BW. Both RPM treatments increased milk fat percentage ($P = 0.02$) and tended to increase milk fat yield ($P = 0.07$) relative to CON, but there was no difference between the RPM sources ($P \geq 0.47$). Milk protein percentage was not different between cows on the RPM-K and RPM-S treatments (3.28%; $P = 0.98$), and both treatments increased protein percentage relative to CON (3.25%; $P = 0.04$). Lactose percentage was reduced by RPM treatments compared with CON ($P = 0.04$) but were not different from one another ($P = 0.78$).

Effect of RPM supplementation on milk yield is variable among studies. Often, no differences are observed in milk yield with supplemental RPM in individual studies (Overton et al., 1998; Ordway et al., 2009; Toledo et al., 2021), but a meta-analysis by Zanton et al. (2014) reported a tendency for an increase in milk yield dependent on the particular source of RPM. Thus, the lack of effect of RPM on milk yield in the present study was not unexpected.

The observed gains in milk protein percentage with RPM supplementation were consistent with the literature. Specifically, both duodenal infusion of Met as well as dietary supplementation with RPM have been found to increase milk protein percentage (Socha et al., 2008; Ordway et al., 2009; Toledo et al., 2021). In their meta-analysis, Zanton et al. (2014) found that supplementation with RPM-S (containing a mean of 16.3 g/d Met and supplying

Table 2. Intake and production responses for all cows that were retained in the final model (multiparous, n = 22, and primiparous, n = 4), which were provided a control diet deficient in MP methionine by approximately 17 g (CON) or the control diet supplemented with either 14 g/d of KESSENT M[1] (RPM-K) or Smartamine M[2] (RPM-S)

	Treatment				Contrast *P*-value[3]	
Item	CON	RPM-S	RPM-K	SEM	RPM	Source
DMI, kg/d	26.3	26.6	26.2	0.8	0.61	0.02
Milk, kg/d	38.3	38.4	38.1	1.4	0.85	0.35
Fat, %	3.60	3.66	3.69	0.17	0.02	0.47
Fat, kg/d	1.37	1.40	1.39	0.06	0.07	0.74
Protein, %	3.25	3.28	3.28	0.10	0.04	0.98
Protein, kg/d	1.24	1.25	1.24	0.05	0.26	0.39
Lactose, %	4.91	4.89	4.88	0.03	0.04	0.78
MUN, mg/dL	8.3	8.4	8.2	0.3	0.86	0.43
ECM, kg/d	39.7	40.3	40.0	1.2	0.18	0.51
ECM/DMI, kg/kg	1.51	1.52	1.53	0.05	0.25	0.37
Somatic cell score[4]	1.91	1.92	2.03	0.40	0.74	0.59
BW, kg	715	718	717	16	0.31	0.54

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

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

[3]Contrasts evaluated the overall effect of providing rumen-protected methionine (RPM; CON vs. RPM-S and RPM-K combined) and the comparison of RPM-S with RPM-K (source; RPM-S vs. RPM-K).

[4]Somatic cell score = $\log_2$(SCC/100,000) + 3.

a mean of 12.6 g/d MP Met) on average increased milk protein percentage by 0.07 percentage units relative to control. The response of the current experiment was lower in magnitude (3.25% for CON vs. 3.28% for RPM-S), but that may have been related to experimental differences in the level of supplementation or degree of Met deficiency in the control diets. However, the lack of difference between RPM-S and RPM-K suggests that both were equally effective in providing MP Met. Despite the increase in milk protein percentage with the RPM treatments, we did not observe an effect on milk protein yield ($P = 0.26$). This was counter to our expectations, as supplementation with RPM typically increases both milk protein percentage and yield (Zanton et al., 2014; Toledo et al., 2021). This was likely due to the lower than typical response in milk protein percentage in the current study as described previously.

Milk fat percentage was increased by RPM treatments when compared with CON ($P = 0.02$), and milk fat yield tended to be increased by RPM ($P = 0.07$). Effect of RPM supplementation on milk fat is more variable than milk protein, with some observing increases (Osorio et al., 2013; Toledo et al., 2021) and others reporting no effects (Ordway et al., 2009; Ardalan et al., 2021). In their meta-analysis, Zanton et al. (2014) found that milk fat yield increased by 1.87 g/d per gram of supplemental MP Met, and the tendency for the increase in milk fat yield with RPM supplementation in the current study is supported by those findings. This effect of Met on milk fat has been suggested to be a result of its function as a methyl donor. Provision of methyl groups can assist in hepatic very-low-density lipoprotein synthesis and export, consequently increasing availability of circulating fatty acids to support milk fat secretion (Emmanuel and Kennelly, 1984; Sharma and Erdman, 1988).

Lactose percentage was reduced for both RPM sources when compared with CON ($P = 0.04$). This was not expected, as most researchers have found no effect of abomasal Met infusion or RPM supplementation on milk lactose concentration (Pisulewski et al., 1996; Batistel et al., 2017; Toledo et al., 2021). In support of our findings, Junior et al. (2021) found a decrease in milk lactose content in response to RPM supplementation. However, Cardoso et al. (2021) found the opposite to be true, with RPM tending to increase milk lactose percentage. Due to the variability in lactose response to RPM supplementation in previously reported studies, we do not expect the decrease in lactose percentage observed with RPM supplementation in the present experiment to be a repeatable effect.

Plasma AA Concentrations

Plasma Met concentration was increased by RPM (46.6 and 46.5 μM for RPM-S and RPM-K, respectively) compared with CON (33.0 μM; $P < 0.001$), with no difference between the 2 RPM sources ($P = 0.97$; Table 3). The RPM treatments did not affect any other AA except for

cystine ($P = 0.02$), Tau ($P = 0.001$), and a tendency for an effect on Gly ($P = 0.06$). Plasma cystine was elevated in cows provided RPM-S or RPM-K (15.7 and 16.2 μ*M*, respectively) relative to CON (15.1 μ*M*), but the RPM sources did not differ from one another ($P = 0.20$). Similarly, RPM increased plasma Tau relative to CON (70.9 and 70.8 μ*M* for RPM-S and RPM-K, respectively, vs. 62.7 μ*M* for CON; $P = 0.001$), but there was no difference between RPM-S and RPM-K ($P = 0.98$). The plasma concentration of Gly tended to be reduced in cows fed RPM-S or RPM-K (289 and 292 μ*M*, respectively) treatments compared with CON (306 μ*M*), with no difference between the RPM sources ($P = 0.77$).

Elevation of plasma free Met is a common response to RPM supplementation (Blum et al., 1999; Rulquin and Kowalczyk, 2003; Ardalan et al., 2021) and was expected in the present experiment. Both RPM treatments increased plasma free Met by approximately 41% relative to CON, with no difference between the RPM sources. Analyzed N contents of the KESSENT M and Smartamine M were 7.28 and 7.59%, respectively, equating to 77.6 and 80.8% Met, respectively. With this data, relative bioavailability of RPM-K can be calculated relative to known 80% bioavailability of RPM-S (Schwab, 1995) as follows:

RPM-K relative bioavailability =
(plasma Met for RPM-K – plasma Met for CON)/
(plasma Met for RPM-S – plasma Met for CON)
× (80.8% Met in RPM-S/77.6% Met in RPM-K)
× 80% bioavailability of RPM-S.

Using the formula, relative bioavailability of Met in RPM-K was calculated as 83% and was similar to that of RPM-S.

In addition, the plasma Met slope response per gram of Met in each product fed was calculated as follows:

Table 3. Plasma AA concentrations (μ*M*) for all cows that were retained in the final model (multiparous, n = 22, and primiparous, n = 4), which were provided a control diet deficient in MP methionine by approximately 17 g (CON) or the control diet supplemented with either 14 g/d of KESSENT M[1] (RPM-K) or Smartamine M[2] (RPM-S)

	Treatment				Contrast *P*-value[3]	
Item	CON	RPM-S	RPM-K	SEM	RPM	Source
3-Methylhistidine	2.97	9.94	3.00	0.21	0.91	0.34
Alanine	301	308	309	13	0.21	0.89
Arginine	79.9	78.0	79.7	3.9	0.65	0.53
Asparagine	56.8	55.7	57.0	2.2	0.79	0.52
Aspartate	5.3	5.1	5.2	0.2	0.25	0.57
Cystine	15.1	15.7	16.2	0.6	0.02	0.20
Glutamate	46.2	45.6	45.6	1.8	0.41	0.95
Glutamine	269	268	276	11	0.66	0.25
Glycine	306	289	292	12	0.06	0.77
Histidine	60.4	61.0	60.6	2.8	0.77	0.81
Isoleucine	130	126	126	6	0.18	0.99
Leucine	162	157	158	8	0.26	0.80
Lysine	96.9	96.6	98.0	5.8	0.91	0.71
Methionine	33.0	46.6	46.5	2.3	0.001	0.97
Phenylalanine	44.2	42.6	43.2	1.7	0.17	0.61
Proline	88.8	88.6	90.2	2.9	0.74	0.50
Serine	81.6	77.7	79.9	2.3	0.24	0.41
Taurine	62.7	70.9	70.8	3.6	0.001	0.98
Threonine	148	144	146	8	0.38	0.68
Tryptophan	37.2	37.2	37.2	1.5	0.97	0.98
Tyrosine	54.9	51.9	52.9	2.1	0.13	0.59
Valine	286	280	280	13	0.34	0.99

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

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

[3]Contrasts evaluated the overall effect of providing rumen-protected methionine (RPM; CON vs. RPM-S and RPM-K combined) and the comparison of RPM-S with RPM-K (source; RPM-S vs. RPM-K).

RPM-S slope response = (plasma Met for RPM-S – plasma Met for CON)/(11.3 g of Met fed in 14 g of RPM-S),

RPM-K slope response = (plasma Met for RPM-K – plasma Met for CON)/(10.9 g of Met fed in 14 g of RPM-K).

Those slope response estimates for each RPM treatment relative to CON were then evaluated in a MIXED model of SAS containing the fixed effect of treatment (RPM-S or RPM-K) and the random effect of cow. Slope response estimates (95% CI) were 1.20 (0.98–1.41) for RPM-S and 1.23 (1.02–1.44) for RPM-K, and these estimates did not differ from one another ($P = 0.77$), again suggesting similar relative bioavailability between RPM-S and RPM-K.

This experiment was not designed to estimate bioavailability, and further testing would be needed to confirm this result. A limitation of the current study is that we measured the N content of the supplements instead of Met content; analyzing for Met would have increased precision. In addition, we only measured plasma Met on 1 d of each period at only 2 times. Daily variability in intake or incomplete consumption of the product dose may have resulted in over- or underestimation of relative bioavailability.

Plasma samples were not subjected to performic acid oxidation to stabilize Cys before analysis; thus, only cystine is reported. However, the increase in plasma cystine in response to both RPM-S and RPM-K is likely in response to the increased Met availability and supports results of others who have found increases in plasma Cys (Berthiaume et al., 2006) or cystine (Pereira et al., 2020). The increase in plasma Tau in response to both RPM-S and RPM-K was similarly expected due to increased Met supply (Berthiaume et al., 2006; Pereira et al., 2020). The tendency for RPM to decrease plasma Gly was unexpected, as this is typically not reported (Berthiaume et al., 2006; Ardalan et al., 2021). However, Pereira et al. (2020) similarly found that RPM tended to decrease Gly.

APPLICATIONS

When post-peak-lactation dairy cows were fed a Met-deficient diet, supplementation with RPM increased milk protein and fat percentages, increased plasma Met concentration, and tended to increase milk fat yield. Both RPM-K and RPM-S caused similar responses in milk composition and plasma Met relative to CON, suggesting similar supplement effectiveness.

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