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Effects of supplementing rumen-protected lysine and methionine during prepartum and postpartum periods on performance of dairy cows

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ABSTRACT

An experiment was conducted to examine effects of prepartum, postpartum, or continuous prepartum and postpartum supply of rumen-protected lysine (RPLys) and rumen-protected methionine (RPMet) on performance and blood metabolites of transition cows. The experiment consisted of a prepartum (3 wk), postpartum (3 wk), and carryover (10 wk) period. Eighty-eight prepartum cows (36 primiparous and 52 multiparous cows) were blocked by parity and expected calving date and assigned to 1 of 4 treatments arranged factorially. Treatments were a prepartum diet (12% crude protein on a dry matter basis) without (Pre−) or with supplemental RPLys (10 g of digestible Lys/cow per day) and RPMet (4 g of digestible Met/cow per day; Pre+) followed by postpartum diets (16% crude protein on a dry matter basis) without (Post−) or with supplemental RPLys (26 g of digestible Lys/cow per day) and RPMet (11 g of digestible Met/cow per day; Post+). Prepartum, only 2 treatments were applied, but postpartum cows received treatments of Pre−Post−, Pre−Post+, Pre+Post−, or Pre+Post+. During the prepartum period, treatment did not affect dry matter intake and body weight. During the postpartum period, milk protein content was greater (3.23 vs. 3.11%) for Post+ compared with Post− independent of prepartum treatment. However, dry matter intake, body weight, milk yield, and yields of milk components were not affected by Post+ versus Post−. No effects of prepartum treatment or interactions between pre- and postpartum treatments were observed on postpartum performance of cows. No effects of pre- and postpartum supplementation of RPLys and RPMet on performance during the carryover period were found except prepartum supplementation of RPLys and RPMet decreased somatic cell count (4.60 vs. 4.83; $\log_{10}$ transformed) compared with Pre− in the postpartum period and this effect continued during the carryover period [i.e., 4.42 and 4.55 ($\log_{10}$ transformed) for Pre+ and Pre−, respectively]. Prepartum supplementation of RPLys and RPMet increased or tended to increase plasma concentration of Lys, Met, and branched-chain AA compared with Pre− in prepartum cows. Cows on Post+ tended to have greater plasma Lys concentration compared with Post−, but plasma Met concentration was not affected. Health events of postpartum cows were not affected by treatments. In conclusion, we did not observe positive effects of supplementing with RPLys and RPMet on performance of prepartum and postpartum cows. However, prepartum supply of RPLys and RPMet may have potential to improve udder health and immune status of fresh cows.

Key words: rumen-protected lysine, rumen-protected methionine, transitioning cows

INTRODUCTION

After parturition, cows experience negative energy and protein balances because nutrient demands for milk production exceed nutrient intake (Drackley, 1999). This causes heavy mobilization of nutrients (fatty acids and AA) to support milk production during early lactation. Because excessive mobilization may cause numerous health disorders after parturition that limit milk production, nutritional management during the transition period is extremely important.

To alleviate heavy AA mobilization after parturition, feeding a high protein diet can be a strategy. For example, Carder and Weiss (2017) observed increased ECM when a high protein diet (high MP) was provided to postpartum cows compared with control (18.4 vs. 16.3% CP in dietary DM). In that study, the high MP supply to postpartum cows decreased plasma 3-methylhistidine concentration compared with control, suggesting lower muscle AA mobilization. However, feeding a high protein diet to cows increases manure N excretion (especially urinary excretion), which can be an environmental concern (Lee et al., 2012b; Carder and Weiss, 2017).

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Feeding a diet that provides a better profile of AA for milk protein synthesis can help alleviate heavy AA mobilization in cows after parturition. The supply of an improved AA profile should increase the efficiency of dietary AA utilization for protein synthesis possibly due to sparing body protein reserves. For example, Carder and Weiss (2017) observed a decrease in plasma 3-methyl-histinine concentration when cows were fed a diet with improved AA profile compared with control. In typical US diets, Lys and Met are assumed to be often limiting (NRC, 2001). Continuous feeding of diets supplemented with rumen-protected Lys (**RPLys**) and Met (**RPMet**) during the prepartum and postpartum periods can increase milk yield and milk protein yield of transition cows (Xu et al., 1998a; Socha et al., 2005). More recently, continuous prepartum and postpartum supply of RPMet significantly increased milk yield and milk protein yield in postpartum cows (Zhou et al., 2016b; Batistel et al., 2017). In these experiments, increased production of cows may have resulted from the supply of improved AA profile to cows prepartum, postpartum, or continuously prepartum and postpartum. However, due to the designs of the experiments, prepartum, postpartum, or prepartum and postpartum effects of rumen-protected amino acid (**RPAA**) supply on production was not possible to be determined separately.

We hypothesized that feeding a diet supplemented with RPLys and RPMet to transition cows will improve milk and milk protein yield. The improved production will be greatest when cows are fed RPLys and RPMet prepartum and postpartum continuously followed by only postpartum supply of RPLys and RPMet. Prepartum supply of RPLys and RPMet may have effects on DMI or metabolic measures before calving, possibly causing positive production or health postpartum. In addition, if RPLys and RPMet supply alleviates body mobilization of AA in postpartum cows, positive production effects may continue after treatments cease.

MATERIALS AND METHODS

All procedures in this project that involved animals were approved by The Ohio State University Institutional Animal Care and Use Committee.

Diets and Experimental Design

Eighty-eight prepartum Holstein cows (36 primiparous and 52 multiparous cows) were used in a randomized complete block design. Cows were blocked according to parity and expected calving date (i.e., 22 blocks of 4 cows). The experiment consisted of a prepartum, postpartum, and carryover period. During the prepartum period, cows in each block were moved at d −21 of anticipated calving to 1 of 2 group pens (i.e., 2 cows to one pen and the other 2 cows to another pen). Two dietary treatments (**Pre−** and **Pre+**) were randomly assigned to the pens: Pre−, a typical prepartum diet; Pre+, the Pre− diet supplemented with rumen-protected (**RP**) Lys [**RPLys**; 10 g of digestible Lys (**dLys**)/cow per day; USA Lysine; Kemin Industries Inc., Des Moines, IA] and Met [**RPMet**; 4 g of digestible Met (**dMet**)/cow per day; MetiPEARL; Kemin Industries Inc.; Table 1]. At d −14 before anticipated calving, cows were moved to individual box stalls and remained on their corresponding diets (Pre− or Pre+) and individual daily intakes were measured. After calving, cows remained in the individual box stalls for about 2 d and then moved to individual tiestalls. Postpartum diets started after calving, but production measurements did not start until 2 DIM (when cows were moved to tiestalls). At calving all cows were fed a typical fresh cow diet (Table 2), but half of the cows within each prepartum treatment group were supplemented with RPLys (26 g of dLys/cow per day) and RPMet (11 g of dMet/cow per day; **Post+**) and half were not supplemented (**Post−**). Therefore, cows in each block were on Pre−Post−, Pre−Post+, Pre+Post−, and Pre+Post+. During the postpartum period, individual intake and production were monitored until 22 DIM. From 23 to 91 DIM, all cows were moved to group pens and received a common lactation diet (Table 3) without supplementation of RPLys and RPMet (carryover period). Although milk yield and milk composition from individual cows were recorded, individual intake was not measured during the carryover period.

Rumen-protected Lys and RPMet were mixed with the corresponding concentrates (i.e., concentrates of Pre+ and Post+). The concentrates were mixed with forages daily and TMR was fed to cows ad libitum. The inclusion rate of RPLys and RPMet was chosen to provide dLys and dMet at about 7.2 and 2.4% of MP, respectively, during the prepartum and postpartum period (NRC, 2001) so that the ratio of dLys to dMet was close to 3.0. Studies on AA supply to prepartum cows are scarce, and to our knowledge, no information for optimal Lys and Met supply to prepartum cows is available. Therefore, the inclusion rates of RPLys and RPMet used had a consistent proportion of dLys and dMet in MP (NRC, 2001) in the prepartum and postpartum periods. The supply of digestible Lys and Met from RPLys and RPMet, respectively, was assumed to be 42 and 29%, respectively (Lys or Met content × rumen bypass rate × absorption rate in the small intestine) according to the manufacturer's information. The

requirement of MP, dLys (MP g/d × 0.072), and dMet (MP g/d × 0.024) in Tables 1 and 2 was calculated using actual DMI and production of cows.

In all periods, diets were prepared as TMR and fed to cows once daily ad libitum (about 5% refusal) with free access to water. Cows were milked in the milking parlor twice a day at approximately 12-h intervals.

Sampling Procedures and Laboratory Analyses

Forages were sampled weekly and assayed for DM (100°C for 24 h) to adjust diets. Individual feeds (forages and concentrates) were sampled weekly and composited into monthly samples for nutrient analyses. The composited samples were dried at 55°C for 48 h and ground through a 1-mm screen (Wiley Mill, Arthur H. Thomas, Philadelphia, PA). Feed samples were analyzed for DM (100°C for 48 h), ash (600°C muffle oven overnight), NDF using sodium sulfite and amylase (Ankom Fiber Analyzer, Ankom Technology, Fairport, NY), CP (Kjeldahl N × 6.25; AOAC International, 2000; 984.13.4.09), starch (Weiss and Wyatt, 2000), and major minerals by inductively coupled plasma spectrometry after ashing at 535°C followed by digestion with 15% nitric acid. Orts were sampled from each cow once weekly while cows were in individual stalls and assayed for DM (100°C oven for 48 h) to calculate DMI.

Feed offered and refused were recorded daily when cows were in individual stalls during the prepartum and postpartum period, but not when cows were in group pens during the prepartum and carryover period. Milk yields were recorded daily (a.m. and p.m.) for all cows after calving. Milk samples (a.m. and p.m.) were collected from individual cows weekly and analyzed for fat, true protein, lactose (B2000 Infrared Analyzer, Bentley Instruments, Chaska, MN), and MUN (Skalar SAN Plus, Skalar Inc., Norcross, GA) by DHI Cooperative Inc. (Columbus, OH).

Cows were weighed on d 10 before anticipated calving and 3, 10, and 21 DIM while in tiestalls. Body condition scores (scale 1 to 5; NRC, 2001) were recorded for individual cows on d 21 before calving, and d 3, 21, 50, and 90 after calving by 3 trained persons. All calves were weighed at birth. Female calves were also weighed at d 50 of age. No treatments were imposed on calves.

Blood samples were collected from the tail vein of cows into heparinized tubes (BD Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ) on d 3 before anticipated calving and 12 and 22 d of lactation. Plasma was separated immediately and frozen at −20°C until analysis. Plasma samples were analyzed for BHB (β-hydroxybutyrate Liquicolor No. 2440, Stanbio Laboratory, Boerne, TX) and fatty acids [NEFA-HR(2), Wako Chemicals, Richmond, VA]. Twelve cows per treatment (8 multiparous and 4 primiparous cows) were randomly selected and a subsample of plasma on d −3 before anticipated calving and on d 22 of lactation was analyzed for concentrations of urea, individual AA, 1-methylhistidine, 3-methylhistidine, and carnosine

Table 1. Feed ingredients and chemical composition of diets fed to prepartum cows

Item	Diet[1]	
	Pre−	Pre+
Ingredient, DM %		
Corn silage[2]	32.0	32.1
Grass balage[3]	23.9	24.1
Grass hay[4]	24.3	24.1
Corn grain, ground	3.5	3.4
Soybean meal, 49% CP	4.9	4.5
Corn gluten meal	2.5	2.4
Animate[5]	1.7	1.7
Soybean hulls	4.9	4.9
Fat true energy[6]	0.18	0.18
Trace mineral salts[7]	0.17	0.17
Vitamin and mineral premix[8]	2.05	2.04
USA Lysine[9]	0	0.24
MetiPEARL[9]	0	0.14
Nutrient composition, % of DM		
DM, % (as fed)	54.9	54.7
CP	12.2	12.2
NDF	52.0	52.2
NE_L, Mcal/kg	1.46	1.46
Ca	1.36	1.32
P	0.35	0.34
K	2.06	2.06
Mg	0.39	0.40
MP supply[10]		
MP supply, g/d	822	813
MP balance, g/d	54	48
dLys supply, g/d	53	61
dLys, % of MP	6.45	7.48
dMet supply, g/d	16	20
dMet, % of MP	2.00	2.46
dLys:dMet	3.22	3.04

[1]Pre− = prepartum diet; Pre+ = the prepartum diet supplemented with rumen-protected Lys and Met.

[2]DM, 32.2% (as-is basis); CP, 8.3% (DM basis); NDF, 39.7% (DM basis); starch, 32.1% (DM basis).

[3]DM, 66.8% (as-is basis); CP, 8.6% (DM basis); NDF, 70.7% (DM basis).

[4]DM, 89.0% (as-is basis); CP, 7.1% (DM basis); NDF, 72.3% (DM basis).

[5]Phibro Animal Health Corp., Teaneck, NJ.

[6]Energy Booster 100; Milk Specialties, Eden Prairie, MN.

[7]NaCl, 93%; Zn, 3,500 mg/kg; Mn, 2,800 mg/kg; Fe, 1,750 mg/kg; Cu, 350 mg/kg; I, 70 mg/kg, Co, 70 mg/kg (as-is basis).

[8]Magnesium sulfate, 0.42% (dietary DM basis); limestone, 0.75%; sodium selenate, 0.15%; vitamin A (30,000 IU/g), 0.02%; vitamin D (3,000 IU/g), 0.06%; vitamin E (44 IU/g), 0.18%; biotin (220 μg/g), 0.46%; copper sulfate, 0.002%.

[9]USA Lysine (Kemin Industries Inc., Des Moines, IA): 55% Lys, 85% rumen bypass, 90% RUP digestibility; MetiPEARL (Kemin Industries Inc.): 48% Met, 66% rumen bypass, 90% RUP digestibility.

[10]Estimated according to NRC (2001) using actual DMI of cows. d = digestible.

(University of Missouri–Columbia, Agricultural Experiment Station Chemical Laboratory; Deyl et al., 1986; Fekkes, 1996). Any health issues that occurred during the experiment were recorded.

Table 2. Ingredients and chemical composition of diets fed to postpartum cows

Item	Diet[1]	
	Post−	Post+
Ingredient, DM %		
Corn silage[2]	32.9	32.6
Alfalfa silage[3]	14.3	14.2
Alfalfa hay[4]	5.1	5.1
Cottonseed, whole	4.0	4.1
Corn grain, ground	23.2	23.4
Soybean meal, 49% CP	8.7	8.2
Corn gluten meal	2.5	2.5
Soybean, hulls	6.1	6.1
Fat true energy[5]	0.88	0.89
Trace mineral salt[6]	0.77	0.78
Vitamin and mineral premix[7]	1.63	1.63
USA Lysine[8]	0.00	0.36
MetiPEARL[8]	0.00	0.22
Nutrient composition, % of DM		
DM, % (as fed)	51.9	51.6
CP	16.1	16.1
NDF	31.8	31.4
Starch	28.4	28.1
NE_L,[9] Mcal/kg	1.69	1.70
Ca	0.99	1.03
P	0.44	0.44
K	1.58	1.56
Mg	0.22	0.22
Lys and Met supply[9]		
MP supply, g/d	2,007	2,043
MP balance, g/d	−252	−263
dLys supply, g/d	121	144
dLys required, g/d	145	147
dLys balance, g/d	−24	−3
dLys, % of MP	6.03	7.07
dMet supply, g/d	38	48
dMet required, g/d	48	49
dMet balance, g/d	−10	−1
dMet, % of MP	1.89	2.34
dLys:dMet	3.24	3.02

[1]Post− = postpartum diet; Post+ = the postpartum diet supplemented with rumen-protected Lys and Met.

[2]DM, 32.2% (as-is basis); CP, 8.3% (DM basis); NDF, 39.7% (DM basis); starch, 32.1% (DM basis).

[3]DM, 41.5% (as-is basis); CP, 17.7% (DM basis); NDF, 42.0% (DM basis).

[4]DM, 84.7% (as-is basis); CP, 16.5% (DM basis); NDF, 47.9% (DM basis).

[5]Energy Booster 100; Milk Specialties, Eden Prairie, MN.

[6]NaCl, 93%; Zn, 3,500 mg/kg; Mn, 2,800 mg/kg; Fe, 1,750 mg/kg; Cu, 350 mg/kg; I, 70 mg/kg, Co, 70 mg/kg (as-is basis).

[7]Limestone, 0.81% (dietary DM basis); sodium selenate (200 mg/kg), 0.15%; vitamin A, 0.02%; vitamin D, 0.06%; vitamin E, 0.07%; calcium phosphate (di-), 0.5%; zinc sulfate, 0.004%; copper sulfate, 0.002%; magnesium oxide, 0.01%.

[8]USA Lysine (Kemin Industries Inc., Des Moines, IA): 55% Lys, 85% rumen bypass, 90% RUP digestibility; MetiPEARL (Kemin Industries Inc.): 48% Met, 66% rumen bypass, 90% RUP digestibility.

[9]Estimated according to NRC (2001) using actual DMI and production of cows. d = digestible.

Statistical Analyses

During the postpartum period, 2 cows on Pre−Post+ had displaced abomasum and were removed from the experiment. Therefore, 20 cows for Pre−Post+ and 22 cows for Pre−Post−, Pre+Post−, and Pre+Post+ were used for data analysis during the postpartum and carryover period. All cows (44 cows per treatment; Pre− and Pre+) in the prepartum period were used for data analysis.

All data were analyzed using the MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). Data of prepartum BW, blood fatty acids, BHB, and AA concentrations, calf BW at birth (all calves) and 50 d in age (only female) were analyzed with a model that included the fixed effects of treatment (Pre− vs. Pre+), parity (first gestation vs. multiparous), interaction of treatment by parity, and days on treatment before calving as a covariate and the random effects

Table 3. Ingredients and chemical composition of a common diet fed during the carryover period

Item	Value
Ingredient, DM %	
Corn silage[1]	37.0
Alfalfa silage[2]	10.0
Alfalfa hay[3]	5.0
Cottonseed, whole	8.0
Corn grain, ground	22.1
Soybean meal, 49% CP	9.1
AminoPlus[4]	2.8
Soybean, hulls	2.8
Fat true energy[5]	0.43
Limestone	0.54
Trace mineral salt[6]	0.65
Vitamin and mineral premix[7]	1.58
Nutrient composition, % of DM	
DM, % (as fed)	62.6
CP	16.1
NDF	31.6
Starch	28.4
Ca	0.92
P	0.42
K	1.91
Mg	0.32

[1]DM, 32.2% (as-is basis); CP, 8.3% (DM basis); NDF, 39.7% (DM basis); starch, 32.1% (DM basis).

[2]DM, 41.5% (as-is basis); CP, 17.7% (DM basis); NDF, 42.0% (DM basis).

[3]DM, 84.7% (as-is basis); CP, 16.5% (DM basis); NDF, 47.9% (DM basis).

[4]Ag Processing Inc., Omaha, NE.

[5]Energy Booster 100; Milk Specialties, Eden Prairie, MN.

[6]NaCl, 93%; Zn, 3,500 mg/kg; Mn, 2,800 mg/kg; Fe, 1,750 mg/kg; Cu, 350 mg/kg; I, 70 mg/kg, Co, 70 mg/kg (as-is basis).

[7]DCAD Plus (Arm & Hammer Animal Nutrition, Princeton, NJ), 0.65% (dietary DM basis); sodium selenate (200 mg/kg), 0.16%; vitamin A (30,000 IU/g), 0.01%; vitamin D (3,000 IU/g), 0.04%; vitamin E (44 IU/g), 0.05%; biotin (220 μg/g), 0.28%; calcium phosphate (di-), 0.20%; Zinpro 120 (Eden Prairie, MN), 0.02%; magnesium oxide, 0.17%; copper sulfate, 0.002%; cobalt carbonate, 0.001%.

of block nested within parity. Prepartum daily DMI was analyzed using the same model except that day, all 2- and 3-way interactions among treatment, parity, and day as fixed effects were included in the model (none of dependent variables had an interaction between parity and independent variables). Day was used as repeated measures with the auto-regressive covariance structure based on the lowest Bayesian information criterion.

Postpartum data of daily DMI, milk yield, and weekly milk composition were statistically analyzed using the model that included the fixed effects of 2 main effects (pre- and postsupplementation), interaction between pre- and postsupplementation, parity, day or week, all the interactions among treatments, parity, and time, and days on treatment before calving as a covariate and the random effects of block nested within parity. Repeated measures with day or week were included with the auto-regressive covariance structure based on the lowest Bayesian information criterion. Data of BW, BW change, and plasma fatty acid and BHB concentrations were also analyzed using the same model except that day of sampling was used as repeated measures. Postpartum plasma AA concentration was analyzed using the same model without repeated measures (data from each day were analyzed separately). Health records were analyzed using the same model except that time effect was not included and the option of chi-square was used to analyze disease occurrence. Production data during the carryover and entire experimental period were analyzed using the same model as for production above except that data of daily milk yield and milk composition were averaged by week within cow and weekly data were analyzed with week as repeated measures. Data of SCC were $\log_{10}$ transformed before analysis.

All denominator degrees of freedom were adjusted using the Kenward-Roger option. Due to unbalanced replication of treatments, the highest standard error of the mean is reported for each dependent variable. Statistical differences were declared at $P < 0.05$. Differences between treatments with $0.05 < P < 0.10$ were considered as a trend toward significance. Data are presented as least squares means.

RESULTS

Dietary Composition, Digestible Lys and Met Supply, and Health of Cows

Nutrient composition of prepartum or postpartum diets were essentially the same between treatments except for Lys and Met (Tables 1 and 2). Estimated DMI and production used to formulate the diets (NRC, 2001) were close to actual DMI and production except that actual DMI of prepartum cows was 2 kg lower than estimated. Supplementation of the prepartum diet with RPLys and RPMet (i.e., Pre+) increased the estimated supply of digestible Lys and Met by 15 and 20%, respectively, and resulted in a decrease in the ratio of proportion of dLys to dMet in MP from 3.22 to 3.04 compared with Pre− (Table 1). During the postpartum period (~21 DIM), inclusion of RPLys and RPMet in the postpartum diet (i.e., Post+) also increased the estimated supply of digestible Lys and Met by 20 and 26%, respectively, which lowered the ratio of dLys:dMet (% in MP) from 3.24 to 3.02 compared with Post− (Table 2). Treatment did not affect disease occurrence and total days that cows received veterinary treatments (Table 4).

Prepartum Period

Supplementing the prepartum diet with RPLys and RPMet did not affect ($P \geq 0.44$) DMI and BW of prepartum cows (Table 5). During the prepartum period, DMI of cows decreased (time effect, $P < 0.01$; data not shown) as calving approached, but no time by treatment interaction was observed. Blood plasma fatty acids and BHB did not differ ($P \geq 0.73$) between prepartum treatments, and calf BW at birth and at d 50 was not affected ($P \geq 0.57$) by supplementation of RPLys and RPMet. None of dependent variables had an interaction between parity and independent variables (data not shown).

Postpartum Period (3 to 21 DIM)

Dry matter intake and most production data (milk yield and milk components) were affected by time ($P < 0.05$; data not shown) as lactation progressed. During the first 3 wk of the postpartum period, only a few measures were significantly or tended to be different among treatments (Table 6). Milk protein content was greater (3.23 vs. 3.11%; $P = 0.01$) for Post+ compared with Post−, but it was not affected ($P = 0.55$) by prepartum treatment (Figure 1). Prepartum supplementation of RPLys and RPMet decreased (4.60 vs. 4.83; $P = 0.01$) SCC compared with Pre−, but it was not affected ($P = 0.24$) by postpartum treatment. Plasma prepartum BHB concentration were not affected by treatment (Table 5), but postpartum concentration tended to be lower (657 vs. 838 μmol/L; $P = 0.051$) for Post+ treatments than Post− treatments. Dry matter intake, milk yield, feed efficiency, milk composition, milk MUN, and plasma fatty acids were not affected ($P \geq 0.12$) by prepartum and postpartum supplementation of RPLys and RPMet. No interaction between Pre and Post were

Table 4. Effects of feeding supplemental rumen-protected Lys and Met to prepartum and postpartum cows on clinical health events during the first 22 d of lactation

	Diet[1]						
	Pre−		Pre+		*P*-value[2]		
Item	Post−	Post+	Post−	Post+	Pre	Post	Int
No. of cows	22	22	22	22			
No. of cows removed[3]	—	2	—	—			
Total cow-days treated[4]	35	4	21	47			
Days treated per cow	1.30	0.70	0.60	1.49	0.92	0.73	0.09
Type of treatment, no.							
Ketosis	2	—	3	2	0.26	0.34	0.83
Hypocalcemia	—	—	—	1	0.41	0.33	0.39
Metritis	3	3	3	5	0.52	0.23	0.39
Retained placenta	2	1	1	2	0.71	0.79	0.52
Mastitis[5]	3	1	3	3	0.79	0.66	0.30
Displaced abomasum	—	2	—	1	0.14	0.24	0.13
No. of cows with at least 1 disease	9	6	7	9	0.80	0.84	0.33

[1]Pre− = prepartum diet; Pre+ = the prepartum diet supplemented with rumen-protected Lys and Met; Post− = postpartum diet; Post+ = the postpartum diet supplemented with rumen-protected Lys and Met.

[2]Pre = Pre− versus Pre+; Post = Post− versus Post+; Int = interaction between Pre and Post.

[3]Right displaced abomasum.

[4]Cows received treatments orally or injected for a disease-related incidence. Cows may have received multiple types of treatments during a given treatment day.

[5]Clinical mastitis.

observed in most variables except there was a trend for an interaction ($P = 0.06$) in milk yield per unit of DMI (kg/kg). However, no interaction was observed for ECM per unit of DMI (kg/kg; Table 6). None of dependent variables had an interaction between parity and independent variables (data not shown).

Carryover Period (22 to 91 DIM) and Whole Experimental Period (3 to 91 DIM)

During the carryover period, few production measures were affected by prepartum and postpartum supplementation of RPLys and RPMet (Table 7). Milk lactose content tended to be lower (4.90 vs. 4.93%, $P = 0.06$) for cows on Pre+ versus Pre−. However, milk lactose yield was not affected ($P = 0.73$) by prepartum RPLys and RPMet supply. Milk urea-N concentration was greater (12.3 vs. 11.9 mg/dL; $P = 0.02$) for Pre+ compared with Pre− and milk SCC (log-transformed) was lower (4.42 vs. 4.55, $P = 0.04$) for Pre+ compared with Pre−. Body weights, milk yield, feed efficiency, and milk composition were not different ($P \geq 0.38$) among treatments. No interaction between Pre and Post was observed ($P \geq 0.12$) during the carryover period. None of dependent variables had an interaction between parity and independent variables (data not shown).

Table 5. Effects of feeding supplemental rumen-protected Lys and Met to prepartum cows (last 21 d of gestation) on DMI, BW, blood metabolites, and calf BW

	Diet[1]			
Item	Pre−	Pre+	SEM	*P*-value
DMI, kg/d (14 d in box stalls)	10.1	10.0	0.14	0.44
BW, kg	730	742	11.6	0.49
BCS	3.58	3.59	0.060	0.91
Blood plasma (d −3 of anticipated calving)				
Fatty acids, μEq/mL	239	229	29.6	0.81
BHB, μmol/L	455	466	20.7	0.73
>1,200,[2] %	0	0		
Calf BW at birth, kg	42.7	42.8	0.75	0.96
Calf BW at 50 d,[3] kg (only female)	71.3	72.5	1.53	0.57

[1]Pre− = prepartum diet; Pre+ = the prepartum diet supplemented with rumen-protected Lys and Met.

[2]Subclinical ketosis; the number of cows with BHB >1,200 μmol/L ÷ total cows × 100.

[3]Twenty-five and 26 females for Pre− and Pre+, respectively.

Plasma AA Composition

During the prepartum period, supplementing with RPLys and RPMet tended to increase ($P = 0.07$) or increased ($P = 0.01$) plasma concentration of Lys and Met, respectively (Table 8). Among EAA, arginine, threonine, and all branched-chain amino acids (**BCAA**) tended to increase ($P \leq 0.09$) or increased ($P = 0.01$) for Pre+ compared with Pre−. As a result, the concentration of total EAA was greater ($P = 0.01$) for Pre+ versus Pre−. The prepartum supply of RPLys and RPMet had minimal effects on NEAA compared with Pre− where asparagine and tyrosine tended to be greater ($P \leq 0.09$) for Pre+ compared with Pre−. No difference was found for concentrations of urea, methylhistidine, and carnosine in plasma.

During the postpartum period, cows on Pre+ had greater (59.8 vs. 48.7, $P < 0.01$) plasma Lys concentration compared with Pre− and cows on Post+ tended to have greater ($P = 0.09$) plasma Lys concentration compared with Post− (Table 9). However, plasma Met concentration was not affected ($P \geq 0.33$) by Pre+ and Post+ compared with Pre− and Post−, respectively. Plasma Arg concentration increased ($P < 0.01$) or tended to increase ($P = 0.08$) for prepartum and postpartum supplementation of RPLys and RPMet compared with Pre− and Post−, respectively. Plasma leucine, isoleucine, and phenylalanine concentrations increased or tended to increase ($P \leq 0.07$) for Pre+ versus Pre− but were not affected by postpartum supplementation of RPLys and RPMet. Postpartum supply of RPLys and RPMet tended to increase ($P = 0.07$) plasma Trp concentration compared with Post−. As a result, total BCAA and EAA tended to be and were greater ($P \leq 0.07$), respectively, for Pre+ versus Pre− but were not affected by Post+ versus Post−.

Table 6. Effects of feeding supplemental rumen-protected Lys and Met to postpartum cows on BW, intake, production, and blood metabolites from 3 to 22 DIM[1]

	Diet[2]							
	Pre−		Pre+			*P*-value[3]		
Item	Post−	Post+	Post−	Post+	SEM	Pre	Post	Int
BW, kg/d	659	654	659	665	13.7	0.68	0.96	0.66
BW change, kg/d	−2.48	−2.49	−2.32	−2.31	0.305	0.52	0.99	0.99
BCS	3.03	3.19	3.16	3.13	0.089	0.76	0.49	0.31
DMI, kg/d	18.4	17.9	17.9	18.2	0.42	0.95	0.77	0.30
Milk, kg/d	33.9	33.9	34.5	33.8	0.85	0.78	0.63	0.66
ECM, kg/d	37.5	38.0	38.0	38.0	1.23	0.83	0.82	0.79
ECM, kg/kg of DMI	2.01	2.11	2.10	2.06	0.067	0.77	0.62	0.30
Fat, %	4.08	4.23	4.14	4.20	0.123	0.88	0.41	0.71
Protein, %	3.13	3.23	3.08	3.23	0.050	0.55	0.01	0.62
Lactose, %	4.82	4.89	4.83	4.82	0.045	0.52	0.50	0.33
Energy,[4] Mcal/kg	0.75	0.77	0.75	0.76	0.012	0.92	0.18	0.73
Fat, kg/d	1.41	1.43	1.43	1.43	0.059	0.79	0.85	0.81
Protein, kg/d	1.06	1.09	1.06	1.08	0.033	0.99	0.35	0.85
Lactose, kg/d	1.65	1.67	1.67	1.64	0.054	0.96	0.93	0.60
Energy, Mcal/d	25.5	26.0	25.9	25.9	0.86	0.89	0.76	0.74
MUN, mg/dL	11.5	11.1	11.8	11.0	0.39	0.79	0.12	0.59
SCC[5]	4.80	4.85	4.51	4.68	0.094	0.01	0.24	0.52
Median (×1,000/mL)	46	45	30	49				
>400,000[6]	3	2	1	0		0.05	0.50	0.80
Blood plasma[7]								
Fatty acids, μEq/mL	196.8	183.2	224.6	198.1	20.09	0.24	0.28	0.72
BHB, μmol/L	873.5	606.4	803.2	707.1	79.39	0.54	0.051	0.13
>1,200 (12 DIM),[8] %	13.6	0	13.6	9.1				
>1,200 (22 DIM),[8] %	22.7	0	27.2	18.2				

[1]A significant time effect ($P < 0.05$) was observed for all variables except milk protein and lactose yields and MUN, but no time × dietary treatment interactions were observed.

[2]Pre− = prepartum diet; Pre+ = the prepartum diet supplemented with rumen-protected Lys and Met; Post− = postpartum diet; Post+ = the postpartum diet supplemented with rumen-protected Lys and Met.

[3]Pre = Pre− versus Pre+; Post = Post− versus Post+; Int = interaction between Pre and Post.

[4]Calculated (NRC, 2001).

[5]Log-transformed.

[6]Number of cows.

[7]Average values from samples collected at 12 and 22 DIM.

[8]Subclinical ketosis; the number of cows with BHB >1,200 μmol/L ÷ total cows × 100.

Prepartum and postpartum supplementation of RPLys and RPMet had minimal effects on plasma concentrations of NEAA, urea, and methyl histidine.

DISCUSSION

Effects of Prepartum Supply of RPLys and RPMet on Prepartum Performance and Blood Composition

Studies about effects of prepartum supplementation of RPAA on prepartum and postpartum performance of dairy cows are scarce. According to previous limited studies, prepartum supplementation of RPAA might be a potential nutritional strategy for dry cow management in terms of DMI and health (e.g., see the discussion later). However, we did not observe any effects of prepartum supplementation on DMI, BW, and plasma fatty acids and BHB in prepartum cows, which are in agreement with previous studies (Xu et al., 1998b; Osorio et al., 2013; Zhou et al., 2016b). However, responses of DMI to supplemental AA in prepartum cows are variable. Socha et al. (2005) fed a diet supplemented with RPMet or RPLys and RPMet to prepartum cows and no change in prepartum DMI was found. Osorio et al. (2013) also reported no difference in prepartum DMI when cows were fed a diet supplemented with RPMet. In a study by Zhou et al. (2016b), however, prepartum cows fed a diet with RPMet increased DMI by 1.1 kg compared with that without RPMet. Batistel et al. (2017) also observed an increase in DMI by 1.2 kg/d for prepartum cows fed a diet with RPMet. The discrepancy in DMI responses to prepartum supply of RPAA among studies is difficult to explain. Body weights of cows prepartum and postpartum were greater up to 50 and 30 kg (on average), respectively, in the previous studies (Zhou et al., 2016b; Batistel et al., 2017) compared with BW of cows in the current study, probably requiring more nutrients for maintenance. If the cows in the previous studies were under greater deficiency of AA compared with cows in the current study, the degree of DMI response to supplemental Met could be greater. An increase in DMI of cows under deficiency of dietary AA supply was observed when supplemental AA was provided (Lee et al., 2012a). In addition, the major difference between the current study and previous studies (Zhou et al., 2016b; Batistel et al.,

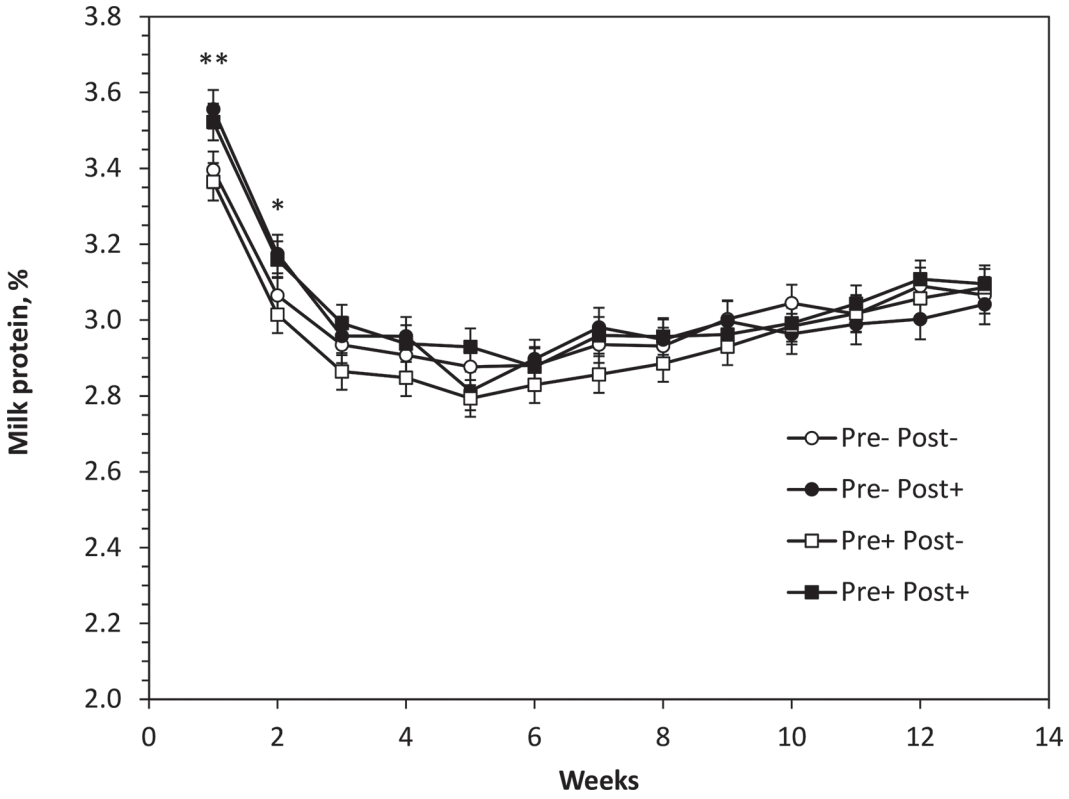

Figure 1. Effects of feeding supplemental rumen-protected Lys and Met to postpartum cows on milk protein content from 3 to 91 DIM (Pre effect, $P = 0.58$; Post effect, $P = 0.09$; interaction of Pre × Post, $P = 0.19$); $^{**}P < 0.05$; $^{*}P < 0.10$. Error bars indicate SEM. Pre− = prepartum diet; Pre+ = the prepartum diet supplemented with rumen-protected Lys and Met; Post− = postpartum diet; Post+ = the postpartum diet supplemented with rumen-protected Lys and Met.

2017) is that we provided RPLys to cows in addition to RPMet so that the ratio of dLys to dMet (% MP) was greater (3.0 vs. 2.8) in the current study versus the previous studies. A meta-analysis indicated that RPLys alone decreased DMI and the combination of RPLys and RPMet did not alter DMI of lactating cows compared with control (no AA supplementation; Robinson, 2010). Furthermore, the commercial RPMet product differed among experiments. A meta-analysis revealed that DMI responses to supplemental RPMet differed between sources of supplemental Met in lactating cows (Zanton et al., 2014). In other meta-analyses, when the source of RPMet was ignored, changes in DMI in response to supplemental RPMet were not observed or minimal (Patton, 2010; Robinson, 2010). More discussion about DMI responses to RPAA will follow later.

Information about plasma AA composition in prepartum cows fed supplemental RPAA is scarce. Supplementing with RPLys and RPMet increased plasma Lys and Met concentration by 13 and 19%, respectively, for Pre+ compared with Pre−, suggesting that RPLys and RPMet supplementation increased available Lys and Met, respectively, for potential tissue utilization. Interestingly, significant increases in plasma isoleucine, leucine, and valine (BCAA) for Pre+ compared with Pre− were observed. This might be, at least in part, a result of Lys catabolism and then production of BCAA and transamination to various NEAA (Lapierre et al., 2009). The rate of catabolism and transamination of Lys may increase when AA supply exceeds the demands of cows (i.e., nonlactating stage).

Effects of Prepartum Supply of RPLys and RPMet on Postpartum Performance and Blood Composition

One of the main objectives in the current study was to examine responses of postpartum performance to prepartum supplementation of RPLys and RPMet. Dry cow management is important to minimize various health problems that cows may have after parturition (Green et al., 2007), which often occurs because of intensive nutrient mobilization to support nutritional demands for milk production (McArt et al., 2015). Osorio et al. (2013) found a tendency for decreased ketosis by prepartum and postpartum supplementation of RPMet. Zhou et al. (2016a,b) reported that cows that received RPMet prepartum and postpartum continuously tended to have less ketosis and few cases of retained placenta and improved liver function and inflammation status during the fresh period. In these experiments, cows that received RPMet prepartum and postpartum increased postpartum DMI, milk

Table 7. Carryover effects of feeding supplemental rumen-protected Lys and Met to prepartum and postpartum cows on BW, intake, and production from 23 to 91 DIM[1]

	Diet[2]							
	Pre−		Pre+			*P*-value[3]		
Item	Post−	Post+	Post−	Post+	SEM	Pre	Post	Int
BW, kg	658	644	659	665	12.7	0.37	0.75	0.41
BW change, kg/d	0.38	0.27	0.38	0.37	0.115	0.61	0.56	0.61
BCS	2.82	2.91	2.97	2.96	0.092	0.24	0.69	0.54
Milk, kg/d	39.8	38.9	39.4	39.4	1.13	0.98	0.68	0.62
ECM, kg/d	42.2	41.2	41.8	41.7	1.17	0.96	0.56	0.66
Fat, %	3.89	3.84	3.93	3.87	0.069	0.51	0.38	0.95
Protein, %	2.98	2.96	2.93	2.99	0.025	0.68	0.40	0.13
Lactose, %	4.94	4.92	4.90	4.90	0.022	0.06	0.47	0.47
Energy,[4] Mcal/kg	0.72	0.72	0.72	0.72	0.007	0.74	0.49	0.72
Fat, kg/d	1.56	1.51	1.56	1.53	0.051	0.81	0.39	0.83
Protein, kg/d	1.18	1.16	1.15	1.17	0.030	0.76	0.94	0.40
Lactose, kg/d	1.97	1.92	1.92	1.93	0.054	0.73	0.64	0.57
Energy, Mcal/d	28.9	28.1	28.5	28.4	0.79	0.96	0.54	0.64
MUN, mg/dL	11.7	12.0	12.5	12.1	0.26	0.02	0.96	0.11
SCC[5]	4.45	4.64	4.42	4.42	0.073	0.04	0.14	0.14
Median (×1,000/mL)	29	34	31	29				
>400,000[6]	0	1	0	1		0.73	0.13	0.74

[1]Cows were fed a common diet in group pens without supplemental rumen-protected Lys and Met.

[2]Pre− = prepartum diet; Pre+ = the prepartum diet supplemented with rumen-protected Lys and Met; Post− = postpartum diet; Post+ = the postpartum diet supplemented with rumen-protected Lys and Met.

[3]Pre = Pre− versus Pre+; Post = Post− versus Post+; Int = interaction between Pre and Post.

[4]Calculated (NRC, 2001).

[5]Log-transformed.

[6]Number of cows.

yield, and milk protein yield as well. However, because cows received RPMet prepartum and postpartum continuously, it was not possible to separate effects of prepartum, postpartum, or continuously prepartum and postpartum supply of RPMet for the improved health and production. Although we did not observe any positive effects of prepartum supplementation of RPAA on health of postpartum cows, the number of cows (22 per treatment) used in the current study may not be adequate in statistical power to evaluate health events. A similar designed study but with fewer cows (9 per treatment) by Kudrna et al. (2009) found similar results. In other studies (Socha et al., 2005; Batistel et al., 2017) where cows were fed RPMet or RPLys and RPMet prepartum and postpartum continuously, no effects on health status were observed and although changes in production during the postpartum period were observed, it is not possible to determine whether the changes were caused by prepartum, postpartum, or continuous pre- and postpartum supplementation of RPMet.

Prepartum supplementation of RPLys and RPMet decreased SCC compared with Pre−, suggesting that supplemental RPAA for prepartum cows may have positive effects on udder health after parturition. A potential positive effect of supplemental RPMet on immune status via enhanced phagocytosis and oxidative functions was observed by Zhou et al. (2016a) where RPMet was fed to cows prepartum and postpartum continuously. In that study, however, SCC was not influenced by the supply of RPMet.

Prepartum supplementation of RPLys and RPMet had similar effects on plasma AA composition for both the prepartum and postpartum period. For example, plasma concentrations of Lys and some of BCAA (Ile and Leu) were or tended to be greater for Pre+ versus Pre− in postpartum cows. This may suggest that prepartum supply of RPLys affected muscle AA composition, which altered plasma AA composition when muscle AA mobilization occurred after parturition. Significant changes in muscle free AA composition (especially Lys and BCAA) were observed in cows when

Table 8. Effects of feeding supplemental rumen-protected Lys and Met to cows on prepartum plasma AA profile (μmol/L) on d −3 of anticipated calving[1]

	Diet[2]			
Item	Pre−	Pre+	SEM	*P*-value
Arg	51.5	56.8	2.17	0.09
His	44.6	47.7	1.76	0.21
Ile	82.6	94.8	3.37	0.01
Leu	127.3	145.7	5.18	0.01
Lys	46.9	52.8	2.30	0.07
Met	18.6	22.2	0.93	0.01
Phe	43.5	45.6	1.33	0.26
Thr	57.2	70.2	5.10	0.07
Trp	31.5	31.7	2.21	0.95
Val	168.2	190.9	6.36	0.01
Branched-chain AA	378.1	431.5	14.14	0.01
Total EAA	672.0	758.5	23.96	0.01
Ala	182.3	194.2	7.34	0.24
Asn	21.3	24.5	1.27	0.07
Asp	5.8	6.3	0.57	0.59
Gln	282.9	288.7	6.55	0.53
Glu	71.0	73.8	2.74	0.46
Gly	247.0	244.6	14.43	0.91
Pro	63.2	67.2	2.73	0.29
Ser	72.6	77.5	3.61	0.33
Tyr	36.8	42.0	2.16	0.09
Total NEAA[3]	982.9	1,018.9	28.82	0.37
Urea, μmol/mL	4.1	4.2	0.21	0.90
1-Methyl histidine[4]	11.0	11.9	0.55	0.24
3-Methyl histidine	9.6	9.2	0.58	0.66
Carnosine	8.0	5.1	8.04	0.77

[1]Twenty-two cows per each treatment were randomly selected to determine plasma AA profile. Blood samples were collected between d −7 to −1 before calving.

[2]Pre− = prepartum diet; Pre+ = the prepartum diet supplemented with rumen-protected Lys and Met.

[3]Parity × treatment, $P = 0.04$; Pre+ tended to be higher ($P = 0.07$) in total NEAA for primiparous cows compared with Pre− and no difference was observed for multiparous cows between Pre+ and Pre−.

[4]Parity × treatment, $P = 0.04$; Pre+ tended to be higher ($P = 0.06$) in 1-methyl histidine for primiparous cows compared with Pre− and no difference was observed for multiparous cows between Pre+ and Pre−.

Table 9. Effects of feeding supplemental rumen-protected Lys and Met to prepartum and postpartum cows on plasma AA profile (μmol/L) on d 22 of lactation[1,2]

	Diet[3]							
	Pre−		Pre+			*P*-value[4]		
Item	Post−	Post+	Post−	Post+	SEM	Pre	Post	Int
Arg	44.9	49.7	53.9	58.9	2.76	<0.01	0.08	0.97
His	39.3	39.4	41.4	42.1	2.99	0.42	0.91	0.92
Ile	91.9	95.9	105.8	111.8	7.28	0.04	0.49	0.89
Leu	147.0	150.0	160.2	177.6	11.06	0.07	0.38	0.51
Lys	46.8	50.6	55.7	63.9	3.61	<0.01	0.09	0.54
Met	21.1	22.4	22.2	23.8	1.51	0.40	0.33	0.95
Phe	42.7	40.7	44.9	45.9	1.91	0.06	0.81	0.43
Thr	81.4	98.4	93.7	97.8	6.63	0.39	0.11	0.31
Trp	25.0	31.7	30.3	32.5	2.43	0.20	0.07	0.35
Val	185.7	199.6	204.4	221.5	14.00	0.14	0.27	0.91
Branched-chain AA	424.6	445.6	470.4	510.9	30.96	0.07	0.32	0.75
Total EAA	726.1	779.8	813.2	876.0	39.33	0.02	0.14	0.91
Ala	207.0	227.7	227.8	215.8	12.97	0.73	0.74	0.21
Asn	33.0	38.5	38.9	39.4	2.32	0.14	0.20	0.29
Asp	6.2	8.4	7.1	7.2	0.67	0.85	0.09	0.11
Gln	239.4	252.2	265.8	246.4	11.81	0.38	0.78	0.17
Glu	55.9	58.2	60.5	61.1	3.14	0.23	0.65	0.78
Gly	352.7	392.6	403.7	407.6	40.06	0.40	0.58	0.65
Pro	80.5	84.8	88.4	87.7	4.98	0.27	0.72	0.61
Ser	90.4	103.9	95.1	101.1	6.64	0.88	0.15	0.57
Tyr	43.1	44.7	48.4	51.4	3.80	0.11	0.55	0.87
Total NEAA	1,108.0	1,211.0	1,235.9	1,217.5	43.08	0.12	0.33	0.16
Urea, μmol/mL	4.1	4.5	4.5	5.0	0.28	0.15	0.12	0.75
1-Methyl histidine	9.0	8.5	7.9	10.3	0.61	0.51	0.11	0.02
3-Methyl histidine	4.2	4.7	3.9	6.3	0.89	0.41	0.11	0.25
Carnosine	6.9	7.0	9.5	7.0	1.53	0.39	0.43	0.41

[1]Twelve cows per each treatment were randomly selected to determine plasma AA profile. Blood samples were collected on d 22 after calving.

[2]DMI (Pre $P = 0.10$, Post $P = 0.43$; Int $P = 0.44$) and milk yields (Pre $P = 0.22$, Post $P = 0.50$; Int $P = 0.87$) among treatments for cows selected for AA analysis were similar.

[3]Pre− = prepartum diet; Pre+ = the prepartum diet supplemented with rumen-protected Lys and Met; Post− = postpartum diet; Post+ = the postpartum diet supplemented with rumen-protected Lys and Met.

[4]Pre = Pre− versus Pre+; Post = Post− versus Post+; Int = interaction between Pre and Post.

dietary AA composition changed significantly (i.e., before parturition and 3 and 15 wk after parturition; Meijer et al., 1995). Lysine and BCAA have been identified as group 2 AA [i.e., after absorption these AA are provided to tissues with minimal oxidation in the liver (Lapierre et al., 2007; Doepel et al., 2009)] and increases in Lys and BCAA supplies may have increased muscle protein synthesis in the current study. However, although this was the case in the current study (i.e., increases in muscle protein synthesis by prepartum supply of RPAA), the degree of AA mobilization in postpartum cows was not altered according to no difference in 3-methylhistidine (Sawada et al., 2013; Carder and Weiss, 2017). No change in plasma Met concentration in postpartum cows for Pre+ versus Pre− may indicate minimal effects of prepartum RPMet supply on muscle Met concentration. Methionine concentration in muscle is much lower than Lys and BCAA (Meijer et al., 1995; NRC, 2001), indicating that relatively minimal effects of prepartum Met supply on the degree of changes in muscle Met storage. It is not clear how Arg was increased for Pre+ compared with Pre−. The increase in Arg likely occurred via increased endogenous synthesis of Arg (Wu et al., 2009) because of no differences in DMI and BW between Pre+ and Pre−. The increased Arg may have been associated with decreased SCC for Pre+ versus Pre− because antioxidant ability and inflammation and immune responses can be improved with Arg supply to mammals (Wu et al., 2009) including dairy cows (Zhao et al., 2018a,b) under stress and inflammatory conditions.

Effects of Postpartum Supply of RPLys and RPMet on Performance and Blood Composition

We did not observe any positive effects of postpartum supply of RPLys and RPMet on DMI and production during the postpartum period (3 to 22 DIM) except that milk protein content increased with supplemental RPLys and RPMet. A few studies with cows fed

RPAA during the fresh period (up to 3 to 4 wk of lactation after parturition) have been conducted and responses have been variable. Osorio et al. (2013) found that fresh cows supplemented with RPMet (up to 30 DIM) increased milk yield and milk protein and fat yields. In that study, DMI of cows fed supplemental RPMet was increased by about 2 kg/d compared with control cows. Similar results were observed by Zhou et al. (2016b). More recently, milk, milk protein, and milk fat yields increased (1 to 60 DIM) when RPMet was provided to postpartum cows (Batistel et al., 2017). Again, DMI was increased for cows fed RPMet by 1.6 kg/d compared with control in this study. In contrast, Socha et al. (2005) reported decreased DMI and numerically lowered milk yield with RPMet during the first 15 wk of lactation compared with control without affecting milk protein yield. In that study, when both RPLys and RPMet were fed to cows, DMI was numerically increased and milk yield and milk protein yield were significantly increased compared with control. Kudrna et al. (2009) found that DMI, milk yield, and milk protein yield were not affected by supplemental RPMet when RPMet was fed to prepartum and postpartum cows until 90 DIM. According to the studies mentioned above, it is likely that positive production responses to supplemental RPAA, if observed, occurred with increased DMI. Generally, feeding supplemental RPLys and RPMet to lactating cows does not affect DMI according to a meta-analysis by Robinson (2010). Another meta-analysis by Patton (2010) found that RPMet alone actually slightly decreased DMI (early- and mid-lactation cows). However, a meta-analysis by Zanton et al. (2014) found that feeding different sources of Met (DL-Met, 2-hydroxy-4-methylthio butanoic acid, Mepron, and Smartamine) had different responses of DMI where DMI was decreased with Mepron (0.25 kg/d; $P = 0.02$) but increased by Smartamine (0.31 kg/d; $P = 0.02$). Although the reason for the discrepancy in DMI responses among Met sources is not known, several possibilities have been addressed (e.g., the level of Met deficiency in a basal diet, presence of co-limiting AA, source of Met, and palatability; Giallongo et al., 2016). Furthermore, most studies (including the current study) did not measure AA bioavailability of RPAA used in the studies; hence, the supply of metabolizable AA may differ from anticipated values. Supplementation of RPAA increased milk protein concentrations similar to what has been often reported (Socha et al., 2005; Robinson, 2010; Osorio et al., 2013; Zanton et al., 2014; Zhou et al., 2016b). In most of these studies, increased milk protein yield coincided with increased milk yield (likely due to increased DMI) and increased milk protein content did not always occur, suggesting importance of increasing volume of milk to increase milk protein yield.

The increases in milk protein content for Post+ occurred in wk 1 and 2 after parturition and this was not observed in wk 3. The degree of Lys and Met deficiency for fresh cows decreased as DMI increased during the postpartum period. However, because milk protein synthesis was similar across treatments (i.e., milk protein yield was not different and milk protein content was likely altered due to changes in milk volume). Indeed, although it was not significantly different, milk yield between Post+ and Post− was 28.9 and 30.2, 34.9 and 35.6, and 37.8 and 37.8 kg/d in wk 1, 2, and 3, respectively (data not shown).

Rumen-protected Met supplementation did not affect plasma Met concentration compared with Post−, whereas RPLys supplementation had a tendency for increased plasma Lys concentration, which supports a meta-analysis by Martineau et al. (2019). The meta-analysis showed that Met as group 1 AA is largely removed by the liver and the response of plasma Met to digestible Met supply is relatively small especially for feeding trials versus infusion trials. In previous studies, increases in plasma Lys and Met concentration after feeding RPLys and RPMet, respectively, to early- and mid-lactating cows were often observed, whereas no changes in plasma Lys and Met concentration with RPLys and RPMet supplementation were also reported (Robinson et al., 2010; Lee et al., 2012b). Plasma AA concentration is the function of AA supply from the gut and utilization or catabolism by the tissues (Meijer et al., 1995). No significant increase in plasma Lys and Met concentration for Post+ versus Post− in the current study does not mean that RPLys and RPMet supplementation (i.e., Post+) used in this study failed to provide additional dLys and dMet because significant increases in plasma Lys and Met were observed in prepartum cows on Pre+. Studies that observed clear increases in plasma Lys and Met with supplemental RPAA often used top-dressing of RPAA on TMR to provide RPAA. When top-dressed, cows likely consume most of RPAA immediately after feeding, leading to a clear peak of corresponding AA in plasma 6 to 12 h after feeding (Toledo et al., 2017). In the current study, RPLys and RPMet were mixed with concentrates and then forages so that the consumption of RPLys and RPMet by cows was relatively slower and consistent throughout the day compared with that when top-dressed. This may have lowered the difference in plasma Lys and Met concentrations between treatments. In addition, minimal or no differences in plasma Lys and Met in the current study may also indicate greater extraction of these AA by tissues. If

this was the case, however, additional dLys and dMet for Post+ compared with Post− were not likely used in the mammary gland for protein synthesis because milk protein yield was not affected in spite of increased milk protein concentration. Utilization of additional AA by extra-mammary tissues rather than mammary tissues was suggested as one of the reasons for the lack of a response to supplemental AA (Nichols et al., 2016; Cant et al., 2018). Then, a question arises: why was additional dLys and dMet not used for milk and protein yield when cows were at the state of high AA demand? As demonstrated by Cant et al. (2018), cows may have been limited by multiple AA, rather than one or 2 AA, causing minimal production responses to Lys and Met supply. A recent meta-analysis by Lean et al. (2018) supports that multiple AA (His, Leu, Trp, and Thr in addition to Lys and Met) are limiting for milk protein synthesis. No changes in plasma BCAA and NEAA concentrations between Post+ and Post− and no interaction between Pre and Post suggests that the catabolism and transamination of prepartum supplementation of Lys that occurred prepartum (i.e., Pre+ vs. Pre−) did not occur or was minimal during the postpartum supply of RPLys. In addition, no evidence was found that prepartum or postpartum supplementation (or both) of RPLys and RPMet improved AA status of fresh cows according to no differences in plasma concentration of 3-methylhistidine and no positive effects on milk protein yield.

Although the concentrations of plasma BHB were in the normal range for all treatments and the difference between Post+ and Post− was not large, a tendency for decreased BHB in plasma for Post+ versus Post− was found. Zhou et al. (2016b) observed fewer occurrence of subclinical ketosis for cows fed RPMet compared with control and suggested potential effects of RPMet on lowering subclinical ketosis. In addition, improved liver function of cows fed RPMet was observed previously (Zhou et al., 2016a; Batistel et al., 2018), which may have affected BHB production for Post+ versus Post−.

Carryover Effects of RPLys and RPMet Supply on Production During the Early Lactation Period

During the carryover phase (23 to 91 DIM) after feeding RPLys and RPMet ceased, no differences in production among treatments were observed. However, the lower SCC for Pre+ versus Pre− that were observed during the fresh period were maintained during the carryover period. As described previously, if supplemental RPLys and RPMet (or increased Arg) improved udder health or immune status, this might have occurred due to prepartum supplementation of RPLys and RPMet because of the lack of differences in SCC between Post+ and Post−. However, on average, SCC for all treatments was low. Zhou et al. (2016a,b) did not find any differences in SCC for cows fed supplemental RPMet prepartum and postpartum although other improvements in health status were found (i.e., decreased occurrence of clinical ketosis and replaced placenta and improved liver function and inflammation status).

CONCLUSIONS

Although supplemental RPLys and RPMet increased estimated dLys and dMet supply and increased significantly or numerically plasma Lys and Met, respectively, DMI, BW, and blood metabolite profiles were not affected by supplemental RPAA prepartum and postpartum. Milk production was not influenced by prepartum and postpartum supplementation of RPLys and RPMet except that milk protein content, but not yield, was increased for cows fed RPAA regardless of the prepartum diets fed. However, prepartum supply of RPLys and RPMet decreased SCC during the fresh and early lactation period. The supply of RPLys and RPMet prepartum and postpartum did not appear to affect the degree of AA mobilization among treatments after parturition. No production effects were observed once treatments ceased.

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