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## Ethyl-cellulose rumen-protected methionine enhances performance during the periparturient period and early lactation in Holstein dairy cows

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### ABSTRACT

The onset of lactation in dairy cows is characterized by severe negative energy and protein balance. Increasing Met availability during this time may improve milk production, hepatic lipid metabolism, and immune function. The aim of this study was to evaluate the effect of feeding ethyl-cellulose rumen-protected methionine (RPM; Mepron, Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany) on the performance of dairy cows during prepartum and early-lactation periods. Sixty multiparous Holstein cows were used in a block design and assigned to either a control or an ethyl-cellulose RPM diet. Ethyl-cellulose RPM was supplied from –28 to 60 d relative to parturition at a rate of 0.09% and 0.10% of dry matter during the prepartum and postpartum periods, respectively. That rate ensured that the ratio of Lys to Met in metabolizable protein was close to 2.8:1. Cows fed ethyl-cellulose RPM had dry matter intakes (DMI) that were 1.2 kg/d greater during the prepartum period and consequently had overall greater cumulative DMI than cows in the control group. Compared with controls, during the fresh period (1–30 d in milk; DIM) feeding ethyl-cellulose RPM increased DMI by 1.7 kg/d, milk yield by 4.1 kg/d, fat yield by 0.17 kg/d, milk protein yield by 0.20 kg/d, 3.5% fat-corrected milk by 4.3 kg/d, and energy-corrected milk by 4.4 kg/d. Although ethyl-cellulose RPM supplementation increased milk protein content by 0.16 percentage units compared with the control during the fresh period, no differences were observed for milk fat, lactose, and milk urea nitrogen concentration. During the high-producing period (31–60 DIM),

cows fed ethyl-cellulose RPM increased DMI and milk yield by 1.45 and 4.4 kg/d, respectively. Ethyl-cellulose RPM also increased fat yield by 0.19 kg/d, milk protein yield by 0.17 kg/d, 3.5% fat-corrected milk by 4.7 kg/d, and energy-corrected milk by 4.8 kg/d compared with controls. Ethyl-cellulose RPM supplementation reduced plasma fatty acids in the fresh period and decreased  $\gamma$ -glutamyl transferase, indicating better liver function. In conclusion, when lysine was adequate, feeding ethyl-cellulose RPM to achieve a ratio close to 2.8:1 in metabolizable protein improved dairy cow performance from parturition through 60 DIM. The greater milk production was, at least in part, driven by the greater voluntary DMI and better liver function.

**Key words:** methionine, milk protein, postpartum, transition period

### INTRODUCTION

Despite the prodigious output of research on nutrition and physiology of dairy cows, the transition period remains a challenging phase during which metabolic disorders continue to occur at economically significant rates (Overton and Waldron, 2004). During the transition period cows experience several stressful events, including regrouping, dietary changes, parturition, and the onset of lactation (Sun et al., 2016). Following parturition, cows enter a period of negative energy and protein balance because they cannot consume enough DM to support the requirements for lactation (NRC, 2001). Although extensive research has been conducted focusing on reducing negative energy balance, less emphasis has been put on overcoming protein and indispensable AA imbalances. Previous research suggests that increasing RUP during late gestation improves subsequent lactation performance (Huyler et al., 1999; Greenfield et al., 2000). The RUP fraction is important as a source of EAA for the mammary gland and other

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tissues, such as liver and skeletal muscle (Osorio et al., 2013). Therefore, an adequate profile and amount of EAA in RUP is crucial for a successful transition into and maintenance of an optimal lactation.

Methionine typically is the first-limiting AA for lactating cows (NRC, 2001). Among the various biological functions besides milk protein synthesis for which Met availability is important, some of the most relevant to the transition period include lipoprotein synthesis in liver, antioxidant synthesis, and synthesis of immune-related proteins (e.g., acute-phase proteins; Osorio et al., 2013; Zhou et al., 2016a). Previous studies have observed beneficial effects of rumen-protected Met (RPM) on performance (Chen et al., 2011; Osorio et al., 2013) and immunometabolic status (Osorio et al., 2014; Zhou et al., 2016a) of dairy cows during the transition period. However, in those studies the effects of RPM on lactation performance were focused only on the immediate postpartum period up to 30 DIM. Knowledge of the potential effect of nutritional strategies that commence in the last few weeks of the dry period (e.g., the close-up) through peak lactation is essential because each additional kilogram of milk secreted at peak production results in approximately 200 kg more milk during the lactation (Roche et al., 2013). Therefore, determining the effects of RPM supplementation not only during the periparturition period but also through peak lactation is of particular importance.

Different sources of RPM might elicit different responses due to inherent differences in either coating system or effectiveness of the coating system (Patton, 2010). Therefore, our general hypothesis was that feeding ethyl-cellulose RPM starting at 4 wk from expected parturition through 60 DIM would improve overall lactation performance not only during the periparturition period but also through peak lactation. The objective of this study was to evaluate the effect of feeding ethyl-cellulose RPM on the performance of dairy cows during the periparturition and early-lactation periods.

## MATERIALS AND METHODS

### *Animal Housing and Care*

The Institutional Animal Care and Use Committee at the University of Illinois (Urbana; protocol no. 14270) approved all experimental procedures. The experiment began on October 25, 2015, and ended on October 10, 2016. All cows were housed in a freestall system equipped with gate system (American Calan Inc., Northwood, NH) during the prepartum period. After parturition, all cows were housed in tiestalls. Cows were fed once daily (1300 h) at 120% of expected intake and milked 3 times daily (at 0600, 1400, and 2200 h). Stan-

dard reproduction and health herd checks and breeding practices were maintained during this study.

### *Design and Treatment Diets*

Sixty multiparous Holstein cows from the University of Illinois Dairy Research Farm were used in a randomized complete block design experiment with 30 cows per treatment. Cows were blocked by the expected parturition day, and the blocks were balanced by parity, previous 305-d milk yield, and BCS. The BCS used to block cows was measured at -30 d before parturition using a 1-to-5 scale (1 = thin, 5 = fat) in increments of 0.25. Cows within each block were randomly assigned to 1 of the 2 treatments. Treatment diets were a basal control diet with no Met supplementation or the basal diet plus ethyl-cellulose RPM (Mepron, Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany). Ethyl-cellulose RPM was supplied from -28 to 60 d relative to parturition at a rate of 0.09 and 0.10% of the DMI of the previous day during the prepartum and postpartum periods, respectively. These target values were based on recent experiments demonstrating a benefit in terms of production performance and health of supplementing RPM to achieve a Lys:Met ratio close to 2.8:1 during the prepartum and postpartum periods (Osorio et al., 2013; Zhou et al., 2016b). Mepron is a commercial rumen-protected source of DL-Met that resists ruminal degradation through an ethyl-cellulose film coating. Pellets measure 1.8 × 3 mm and contain 85% DL-Met. The intestinal digestibility coefficient and rumen bypass of Mepron is 90% (Schwab, 1995) and 80% (Overton et al., 1996); therefore, the cows received 6.1 g of Met available for absorption/10 g of Mepron. During the far-off period (from -45 to -29 d), all cows received the same diet (1.33 Mcal/kg of DM and 13.9% CP) with no RPM. The basal close-up (from -28 d to parturition), fresh (from 1 to 30 d), and high-production (from 31 to 60 d) diets contained 1.47 Mcal/kg of DM and 15.3% CP, 1.67 Mcal/kg of DM and 17.7% CP, and 1.61 Mcal/kg of DM and 17.4% CP, respectively. Diets were mixed daily in a tumble mixer, and ethyl-cellulose RPM was top dressed on the TMR. The ingredient and nutrient compositions of the diets fed are reported in Tables 1 and 2. All rations were formulated to meet cow predicted requirements according to NRC (2001).

### *Data and Sample Collection and Analysis*

Dry matter of individual feed ingredients and diets was determined weekly and used to adjust the DM of the TMR accordingly. Weekly samples of ingredients and TMR were frozen at -20°C and composited monthly for analysis of CP (AOAC International, 2000;

**Table 1.** Ingredient and nutrient composition of far-off (from -45 to -29 d), close-up (from -28 d to parturition), fresh (from 1 to 30 DIM), and high-producing (from 31 to 60 DIM) diets

Ingredient, % of DM	Far-off	Close-up	Fresh	High producing
Alfalfa haylage	—	6.55	7.81	10.8
Corn silage	34.7	26.6	31.0	31.9
Wheat straw	33.7	26.5	3.25	—
Corn grain, ground, dry	—	12.6	22.21	20.7
Cottonseed	—	—	2.17	1.83
Molasses, beet sugar	—	4.03	5.50	4.51
Soybean hulls	15.7	3.46	4.25	9.96
Soybean meal, 48% CP	12.0	7.83	10.1	7.98
Expeller soybean meal <sup>1</sup>	—	5.80	5.16	5.17
Protein supplement <sup>2</sup>	—	0.78	1.81	1.58
Urea	0.46	0.59	0.39	0.40
Soychlor <sup>3</sup>	—	1.23	—	—
Saturated fat supplement <sup>4</sup>	—	—	2.25	2.14
Limestone	—	—	1.41	0.96
Salt	0.40	—	0.02	0.04
Dicalcium phosphate	0.50	0.52	1.17	0.92
Magnesium oxide	—	—	0.08	0.04
Magnesium sulfate	1.90	2.08	0.02	—
Sodium bicarbonate	—	—	0.84	0.59
Mineral vitamin mix <sup>5</sup>	0.40	0.17	0.17	0.20
Vitamin A <sup>6</sup>	—	0.03	0.02	0.02
Vitamin D <sup>7</sup>	—	0.03	—	—
Vitamin E <sup>8</sup>	0.40	0.60	—	—
Biotin <sup>9</sup>	—	0.70	0.42	0.32
Momensin <sup>10</sup>	0.01	—	—	—
Ethyl-cellulose RPM <sup>11</sup>	—	0.09	0.10	0.10

<sup>1</sup>SoyPlus, West Central Soy (Ralston, IA).

<sup>2</sup>ProVAA1 AADvantage, Perdue AgriBusiness (Salisbury, MD).

<sup>3</sup>West Central Soy.

<sup>4</sup>Energy Booster 100, Milk Specialties Global (Eden Prairie, MN).

<sup>5</sup>Contained a minimum of 5% Mg, 10% S, 7.5% K, 2.0% Fe, 3.0% Zn, 3.0% Mn, 5,000 mg of Cu/kg, 250 mg of I/kg, 40 mg of Co/kg, 150 mg of Se/kg, 2,200 kIU of vitamin A/kg, 660 kIU of vitamin D<sub>3</sub>/kg, and 7,700 IU of vitamin E/kg.

<sup>6</sup>Contained 30,000 kIU/kg.

<sup>7</sup>Contained 5,000 kIU/kg.

<sup>8</sup>Contained 44,000 kIU/kg.

<sup>9</sup>ADM Animal Nutrition (Quincy, IL).

<sup>10</sup>Rumensin, Elanco Animal Health (Greenfield, IN).

<sup>11</sup>Ethyl-cellulose rumen-protected methionine, Evonik Nutrition and Care GmbH (Hanau-Wolfgang, Germany). Added only in the treatment group.

method 990.03), NDF with heat-stable  $\alpha$ -amylase and sodium sulfite (Van Soest et al., 1991), ADF (Van Soest et al., 1991), and ether extract (AOAC International, 2000; method 2003.05) by Cumberland Valley Analytical Services (Hagerstown, MD). Evonik Nutrition and Care GmbH analyzed feed ingredients for AA (AOAC International, 1995). The values for NE<sub>L</sub>, RUP, and RDP were predicted using the NRC (2001) model. We used the mean chemical composition of feed ingredients throughout the experiment to evaluate prepartal and postpartal diets using the NRC (2001) model. Feed offered and refused was recorded daily throughout the entire experiment.

Milk production was recorded daily, and consecutive morning, midday, and evening samples were collected once a week, composited in proportion to milk yield,

and stored with preservative at 4°C before analysis of fat, protein, lactose, and MUN by mid-infrared procedures (Dairy Lab Services, Dubuque, IA). Based on milk sample analysis, the 3.5% ECM and FCM were calculated as follows:

$$\begin{aligned} \text{ECM} &= [12.82 \times \text{fat yield (kg)}] \\ &+ [7.13 \times \text{protein yield (kg)}] \\ &+ [0.323 \times \text{milk yield (kg)}]; \end{aligned}$$

$$\begin{aligned} \text{FCM} &= [0.4324 \times \text{milk yield (kg)}] \\ &+ [16.216 \times \text{milk fat (kg)}]. \end{aligned}$$

Body weight and BCS were recorded weekly throughout the entire experiment. Body condition was scored

**Table 2.** Nutrient composition (mean  $\pm$  SD) and diet evaluation using NRC (2001) of prepartal and postpartal diets fed to multiparous Holstein cows supplemented with ethyl-cellulose rumen-protected methionine (RPM)

Item	Far-off	Close-up		Fresh		High-producing	
		Control	Ethyl-cellulose RPM	Control	Ethyl-cellulose RPM	Control	Ethyl-cellulose RPM
Chemical composition, %							
DM							
CP	13.9 $\pm$ 0.25	15.6 $\pm$ 0.32	15.7 $\pm$ 0.32	17.7 $\pm$ 0.36	17.7 $\pm$ 0.36	17.4 $\pm$ 0.36	17.6 $\pm$ 0.36
NDF	54.5 $\pm$ 0.75	40.8 $\pm$ 0.68	40.7 $\pm$ 0.68	29.2 $\pm$ 0.59	29.3 $\pm$ 0.59	31.4 $\pm$ 0.62	31.3 $\pm$ 0.62
ADF	36.9 $\pm$ 0.65	27.5 $\pm$ 0.50	27.4 $\pm$ 0.50	19.5 $\pm$ 0.38	19.6 $\pm$ 0.38	21.5 $\pm$ 0.48	21.4 $\pm$ 0.48
NFC	24.7 $\pm$ 0.72	34.9 $\pm$ 0.81	34.9 $\pm$ 0.81	41.4 $\pm$ 0.55	41.4 $\pm$ 0.55	40.7 $\pm$ 0.54	40.9 $\pm$ 0.54
Ether extract	1.81 $\pm$ 0.04	2.32 $\pm$ 0.05	2.33 $\pm$ 0.05	5.12 $\pm$ 0.14	5.11 $\pm$ 0.14	5.13 $\pm$ 0.14	5.15 $\pm$ 0.14
Calculated using NRC (2001) <sup>1</sup>							
NE <sub>L</sub> , Mcal/kg of DM	1.33	1.47	1.47	1.67	1.67	1.60	1.60
NE <sub>L</sub> allowable milk, kg/d	—	—	—	32.3	34.8	42.8	45.3
MP allowable milk, kg/d	—	—	—	38.8	40.8	49.4	51.7
RDP, % of DM	8.8	9.4	9.4	10.2	10.2	9.9	10
RUP, % of DM	5.1	6.2	6.3	7.4	7.5	7.4	7.6
RDP required, g/d	1,149	1,194	1,196	2,003	2,005	2,505	2,512
RDP supplied, g/d	1,157	1,204	1,203	2,014	2,014	2,508	2,518
RDP balance, g/d	8	10	7	11	9	3	7
RUP required, g/d	131	119	130	1,599	1,802	1,904	2,150
RUP supplied, g/d	668	793	924	1,461	1,611	1,876	2,022
RUP balance, g/d	537	673	794	-138	-191	-28	-128
MP required, g/d	790	808	808	2,543	2,800	3,078	3,200
MP supplied, g/d	1,211	1,363	1,473	2,425	2,640	3,055	3,134
MP balance, g/d	421	555	664	-118	-160	-23	-66
Lys, % of MP	6.74	6.54	6.51	6.40	6.38	6.33	6.29
MP-Lys, g	82	89	89	155	156	194	195
Met, % of MP	1.77	1.73	2.30	1.70	2.24	1.68	2.22
MP-Met, g	21	24	32	41	56	52	68
Lys:Met	3.81:1	3.71:1	2.81:1	3.78:1	2.88:1	3.78:1	2.82:1

<sup>1</sup>The NRC (2001) evaluation of diets was based on final averaged pre- and postpartum DMI, production data, and feed analysis.

on a 5-point scale (1 = thin, 5 = fat) as described by Wildman et al. (1982).

Feed efficiency was calculated by dividing milk yield by DMI, 3.5% ECM by DMI, and FCM by DMI. The energy balance was calculated for each cow using equations from Hardie et al. (2015). Net energy intake was determined using the average weekly DMI multiplied by net energy density of the diet. Net energy of maintenance was calculated as  $NE_M = BW \cdot 0.75 \times 0.080$ . Requirements of milk net energy were calculated as  $NE_L = (0.0929 \times \text{fat } \% + 0.0547 \times \text{protein } \% + 0.0395 \times \text{lactose } \%) \times \text{milk yield}$ . Postpartum energy balance (Mcal/d) was calculated as net energy intake - ( $NE_L + NE_M$ ).

Blood was sampled from the coccygeal vessel before the a.m. feeding in 15 cows per treatment at d -30, -14, 1, 7, 21, 30, and 60 relative to calving date. This subset of cows was chosen based on absence of clinical disease. Samples were collected into evacuated tubes (BD Vacutainer, BD and Co., Franklin Lakes, NJ) containing lithium heparin. After blood collection, samples were placed on ice until centrifugation (~40 min). Plasma was obtained by centrifugation at  $2,000 \times g$  for 30 min at 4°C. Aliquots of plasma were

frozen (-80°C) until further analysis. Glucose (catalog no. 0018250840), urea (catalog no. 0018255440), and  $\gamma$ -glutamyl transferase (**GGT**; catalog no. 0018257640) were analyzed using the IL Test purchased from Instrumentation Laboratory Spa (Werfen Co., Milan, Italy) in the ILAB 600 clinical autoanalyzer (Instrumentation Laboratory, Lexington, MA). Nitric oxide and constituents (nitrite and nitrate) were determined according to methods previously described by Trevisi et al. (2013). Fatty acids and BHB were measured using kits from Wako Chemicals (Richmond, VA) and Randox Laboratories Ltd. (Crumlin, United Kingdom), respectively, following the procedures described previously (Bionaz et al., 2007; Trevisi et al., 2012).

Minerals were extracted by adding 0.3 mL of trichloroacetic acid (10% vol/vol) to 0.3 mL of plasma, and the sample was mixed and centrifuged at  $3,500 \times g$  for 10 min at 4°C. A volume of 0.3 mL of supernatant was added to 2.7 mL of Millipore (Billerica, MA) water and mixed. Concentrations of Ca, Cl, K, Mg, Na, P, and Zn in the final solution were determined with an inductively coupled plasma optical emission spectrometer (ICP-OES 5100, Agilent Technologies, Santa Clara, CA) fitted with a cyclonic chamber in which



samples were introduced with SeaSpray nebulizer (Agilent Technologies). Accuracy of the results was verified using a mineralized solution of SRM 1577b (National Institute of Standards and Technology, Gaithersburg, MD).

Liver was sampled via puncture biopsy (Dann et al., 2005) from 10 cows per treatment. Cows were chosen based on previous 305-d milk yield, BCS, absence of clinical disease, and enrollment for blood samples. Samples were collected from the same cows under local anesthesia at approximately 0800 h on d  $-21$ ,  $-10$ ,  $1$ , and  $10$  relative to parturition. Liver was frozen immediately in liquid N and stored ( $-80^{\circ}\text{C}$ ) until further analysis for concentration of triacylglycerol (**TAG**) as described by Zhou et al. (2016b).

### Statistical Analysis

The production data were analyzed separately for prepartum, fresh (from 1 to 30 d postpartum), and high-producing (from 31 to 60 d postpartum) periods. All weekly data were analyzed using the MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC) according to the following model with repeated measures:

$$Y_{ijkl} = \mu + b_i + M_j + T_k + MT_{jk} + c_l(b_i) + e_{ijkl}$$

where  $Y_{ijkl}$  = dependent continuous variable,  $\mu$  = overall mean,  $b_i$  = random effect of block ( $i = 1-7$ ),  $M_j$  = fixed effect of treatment ( $j = \text{control vs. ethyl-cellulose RPM}$ ),  $T_k$  = fixed effect of time (days or weeks),  $MT_{jk}$  = interaction between treatment and time,  $c_l(b_i)$  = random effect of cow within block, and  $e_{ijkl}$  = residual error. The covariates for parity, previous 305-d milk

yield, and BCS at  $-30$  d relative to parturition were kept in the model for all variables when significant ( $P \leq 0.15$ ). For the analysis of prepartum DMI, the average DMI of individual cows during the far-off period was used as a covariate in the model, whereas plasma data at  $-30$  d relative to parturition was included as a covariate for the blood biomarker analyses. Unless otherwise specified, first-order autoregressive was the covariate structure used for analysis because it resulted in the lowest Bayesian information criterion for most of the variables measured. Normality of the residuals was checked with normal probability and box plots. Treatment differences within week were analyzed using the SLICE option. Cumulative milk yield and DMI were analyzed using the MIXED procedure of SAS using the model described above without time effect. Significance was determined at  $P \leq 0.05$ , and tendencies were determined at  $P \leq 0.10$ .

All cows were in apparent good health at the beginning of the study, and treatment groups were not different in terms of previous 305-d milk yield ( $P = 0.47$ ) or BW ( $P = 0.51$ ) and BCS ( $P = 0.39$ ) at  $-30$  d prepartum. One of the cows in the control group had lameness with traumatic origin at 37 DIM; thus, the data of the last 2 wk and the cumulative milk yield and cumulative DMI during the high-producing period were excluded from the statistical analyses. All other data were included.

## RESULTS

### Health

The incidence of disorders is summarized in Table 3. Ketosis and retained placenta were the most frequent health issues; however, their occurrence was similar between treatments. The incidence of retained placenta was higher (67% of total cases) during July and August 2016.

### Diets

The ingredient composition of the diets is presented in Table 1. The nutrient composition (Table 2) was determined by analyzing each individual feed ingredient for its chemical composition and then entering the feed analysis results into the NRC (2001) model.

### Close-Up DMI

Cows in the ethyl-cellulose RPM group had greater ( $P < 0.01$ ) DMI during the close-up period (average = 1.2 kg/d) and consequently had greater ( $P = 0.01$ ) cumulative DMI compared with the control (Table 4;

**Table 3.** Health incidents (no.) in multiparous Holstein cows fed the control diet or ethyl-cellulose rumen-protected methionine (RPM) during the close-up (from  $-28$  d to parturition), fresh (from 1 to 30 DIM), and high-producing (from 31 to 60 DIM) periods

Item	Control	Ethyl-cellulose RPM
Close-up period		
Lameness	1	0
Postpartum d 1-30		
Ketosis <sup>1</sup>	8	7
Lameness	1	1
Mastitis	1	2
Milk fever	1	0
Retained placenta <sup>2</sup>	7	8
Displaced abomasum	2	1
Postpartum d 31-60		
Lameness (traumatic origin)	0	1
Mastitis	1	0

<sup>1</sup>Defined as cows having large ketone concentration ( $>80$  mg/dL) in urine; tested once a day.

<sup>2</sup>Defined as fetal membranes retained  $\geq 24$  h after calving.

**Table 4.** Effects of ethyl-cellulose rumen-protected methionine (RPM) supplementation to multiparous Holstein dairy cows during the close-up (from -28 d to parturition), fresh (from 1 to 30 DIM), and high-producing (from 31 to 60 DIM) periods on DMI and production

Item	Control	Ethyl-cellulose RPM	SEM	P-value		
				Treatment <sup>1</sup>	Time	Treatment × time
<b>Close-up period</b>						
DMI, kg/d	14.6	15.8	0.13	<0.01	<0.01	0.58
Cumulative DMI, kg	342	382	10.5	0.01	NA <sup>2</sup>	NA
BW, kg	789	784	15.6	0.60	0.68	0.98
BCS	3.76	3.74	0.06	0.70	<0.01	0.58
<b>Fresh period</b>						
DMI, kg/d	16.1	17.7	0.35	<0.01	<0.01	0.71
Cumulative DMI, kg	476	520	10.40	<0.01	NA	NA
Yield, kg/d						
Milk	37.1	41.2	1.48	0.03	<0.01	<0.01
Fat	1.59	1.76	0.07	0.03	<0.01	0.63
Protein	1.22	1.41	0.48	<0.01	0.08	0.95
Lactose	1.86	2.11	0.07	<0.01	0.01	0.29
3.5% FCM	37.3	41.6	1.70	0.01	<0.01	0.68
ECM	37.4	41.7	1.64	<0.01	<0.01	0.75
Cumulative milk yield, kg	1,098	1,208	44.60	0.07	NA	NA
Milk composition, %						
Fat	4.10	4.07	0.11	0.58	<0.01	0.45
Protein	3.19	3.35	0.06	0.04	<0.01	0.68
Lactose	4.73	4.75	0.03	0.48	<0.01	0.93
MUN, mg/dL	13.1	13.3	0.30	0.41	0.70	0.74
Efficiency						
Milk:DMI	2.29	2.31	0.06	0.77	<0.01	0.02
ECM:DMI	2.71	2.75	0.08	0.64	<0.01	0.90
FCM:DMI	2.74	2.82	0.09	0.51	<0.01	0.88
BW, kg	705	700	16.10	0.67	0.02	0.98
BCS	3.16	3.17	0.06	0.65	<0.01	0.13
<b>High-producing period</b>						
DMI, kg/d	20.6	22.1	0.40	0.01	0.05	0.23
Cumulative DMI, kg	613	655	12.8	0.02	NA	NA
Yield, kg/d						
Milk	45.8	50.2	1.70	0.04	0.65	0.98
Fat	1.60	1.79	0.07	0.02	<0.01	0.86
Protein	1.23	1.40	0.05	0.01	0.21	0.83
Lactose	2.20	2.50	0.09	0.01	0.44	0.87
3.5% FCM	45.9	50.6	1.84	0.01	0.11	0.88
ECM	46.0	50.7	1.74	0.01	0.22	0.89
Cumulative milk yield, kg	1,371	1,488	54.50	0.08	NA	NA
Milk composition, %						
Fat	3.62	3.57	0.09	0.70	<0.01	0.93
Protein	2.72	2.87	0.05	0.04	<0.01	0.18
Lactose	4.86	4.89	0.03	0.15	<0.01	0.99
MUN, mg/dL	15.5	15.6	0.23	0.86	0.77	0.82
Efficiency						
Milk:DMI	2.24	2.27	0.08	0.65	<0.01	<0.01
ECM:DMI	2.21	2.23	0.07	0.82	0.04	0.90
FCM:DMI	2.26	2.33	0.07	0.49	0.01	0.89
BW, kg	668	664	16.1	0.67	0.91	0.99
BCS	2.95	2.94	0.06	0.24	0.01	0.98

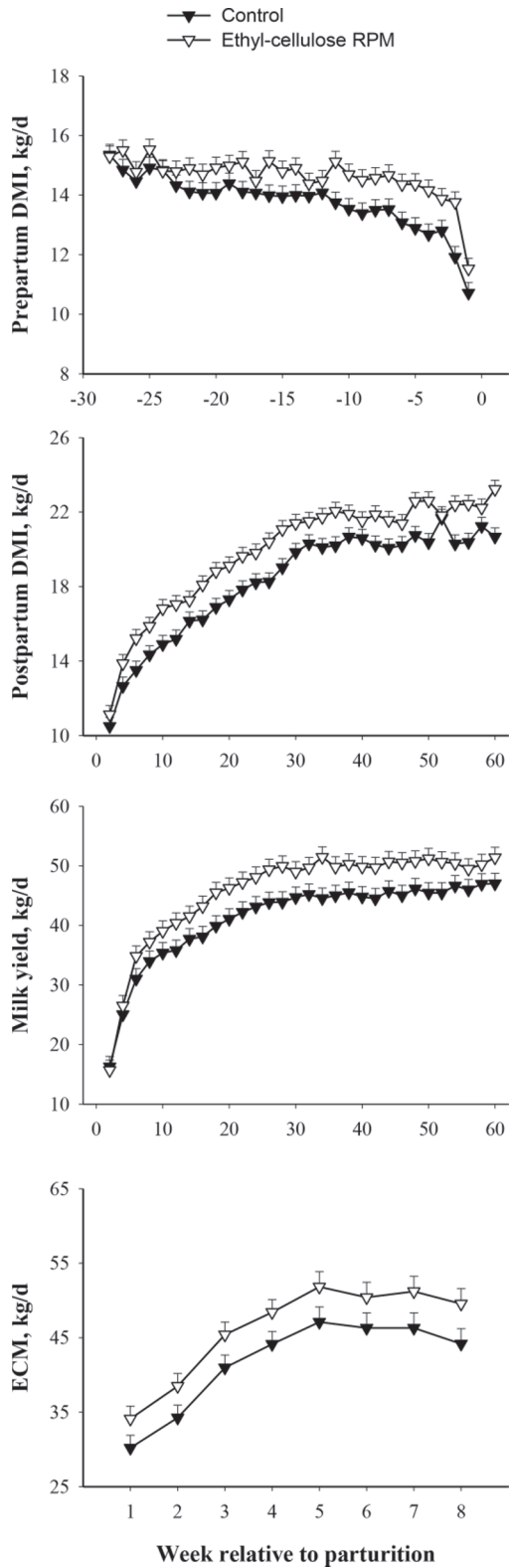
<sup>1</sup>Ethyl-cellulose RPM effect.<sup>2</sup>NA = not applicable.

Figure 1). The DMI in both groups progressively decreased from -10 d to parturition. No differences ( $P > 0.10$ ) between treatments were observed for BW and BCS.

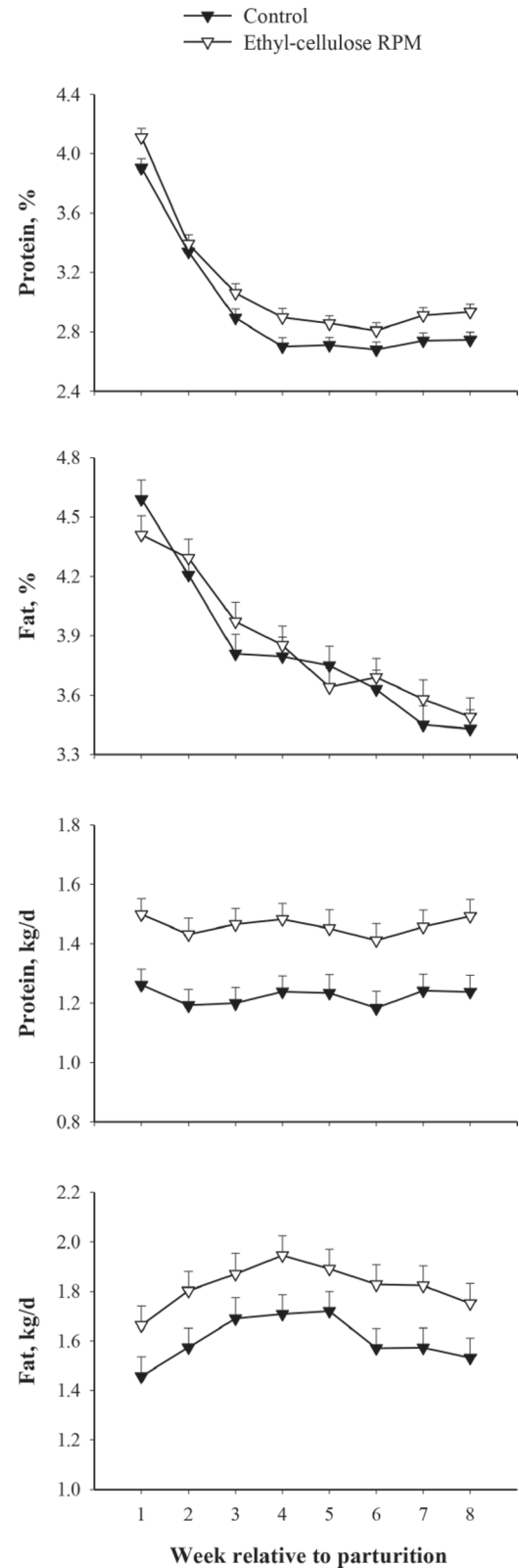
### Fresh Period Production Responses

Ethyl-cellulose RPM supplementation led to greater ( $P < 0.01$ ) DMI (+1.65 kg/d), milk yield (+4.1 kg/d;  $P$

= 0.03), fat yield (+0.17 kg/d;  $P = 0.03$ ), milk protein yield (+0.2 kg/d;  $P < 0.01$ ), milk lactose yield (+0.25 kg/d;  $P = 0.01$ ), 3.5% FCM (+4.3 kg/d;  $P = 0.01$ ), and ECM (+4.4 kg/d;  $P < 0.01$ ; Table 4; Figures 1 and 2). A treatment × time interaction ( $P < 0.01$ ) was observed for milk yield due to a greater increase throughout the experiment in cows supplemented with ethyl-cellulose RPM. In addition, cows in the ethyl-



**Figure 1.** Effects of ethyl-cellulose rumen-protected methionine (RPM) supplementation to multiparous Holstein cows from -28 through 60 DIM on prepartum DMI (last 28 d of pregnancy), postpartum DMI (from 1 to 60 DIM), milk yield, and ECM. Error bars represent SEM.



**Figure 2.** Effects of ethyl-cellulose rumen-protected methionine (RPM) supplementation to multiparous Holstein cows from -28 through 60 DIM on milk fat and milk protein content and yield. Error bars represent SEM.

cellulose RPM group had greater cumulative DMI ( $P < 0.01$ ) and tended ( $P = 0.07$ ) to have greater cumulative milk yield.

Although cows in the ethyl-cellulose RPM group had greater ( $P = 0.04$ ) milk protein content (+0.16 percentage units), no differences ( $P > 0.10$ ) were observed for milk fat, lactose, and MUN. Furthermore, no differences ( $P > 0.10$ ) were observed for feed efficiency, BW, and BCS. As expected, over time both groups of cows experienced increases in DMI and the yield of milk and milk components, but BW and BCS decreased.

### High-Producing Period Responses

Ethyl-cellulose RPM supplementation led to greater ( $P = 0.01$ ) DMI (+1.45 kg/d), milk yield (+4.4 kg/d;  $P = 0.04$ ), fat yield (+0.19 kg/d;  $P = 0.02$ ), milk protein yield (+0.17 kg/d;  $P = 0.01$ ), milk lactose yield (+0.30 kg/d;  $P = 0.01$ ), 3.5% FCM (+4.7 kg/d;  $P = 0.01$ ), and ECM (+4.8 kg/d;  $P = 0.01$ ; Table 4; Figure 1 and 2). In addition, cows in ethyl-cellulose RPM had greater cumulative DMI ( $P = 0.02$ ) and tended to have greater cumulative milk yield ( $P = 0.08$ ). Although cows in ethyl-cellulose RPM had greater ( $P = 0.04$ ) milk protein content (+0.15 percentage units), no differences ( $P > 0.10$ ) were observed for milk fat, lactose, and MUN. Furthermore, no differences ( $P > 0.10$ ) were observed for feed efficiency, BW, and BCS.

### Energy Balance

During the fresh period, cows in the ethyl-cellulose RPM group had net energy intakes that were 2.9 Mcal/d greater and milk energy outputs that were 4 Mcal/d greater compared with controls ( $P < 0.01$ ; Table 5). No differences ( $P > 0.10$ ) in energy partitioned to maintenance and energy balance were observed. As lactation

progressed, cows increased energy intake, and negative energy balance was ameliorated (Figure 3).

In the high-producing period, ethyl-cellulose RPM cows had greater energy intake (+2.4 Mcal/d;  $P < 0.01$ ) and milk energy output (+2.8 Mcal/d;  $P = 0.05$ ). However, no differences ( $P > 0.10$ ) in energy allocated to maintenance and energy balance were observed between treatments.

### Biomarkers in Plasma and Liver TAG

Compared with controls, ethyl-cellulose RPM cows had lower concentrations of GGT ( $P = 0.03$ ) and tended to have lower concentrations of fatty acids ( $P = 0.08$ ). No differences ( $P > 0.10$ ) between groups were observed for blood glucose, BHB, urea, nitric oxide,  $\text{NO}_2$ ,  $\text{NO}_3$ , liver TAG, and mineral concentrations (Table 6; Figure 4; Supplemental Figures S1 and S2, <https://doi.org/10.3168/jds.2017-11631>).

## DISCUSSION

### Nutrient Composition and NRC Evaluation of Diets

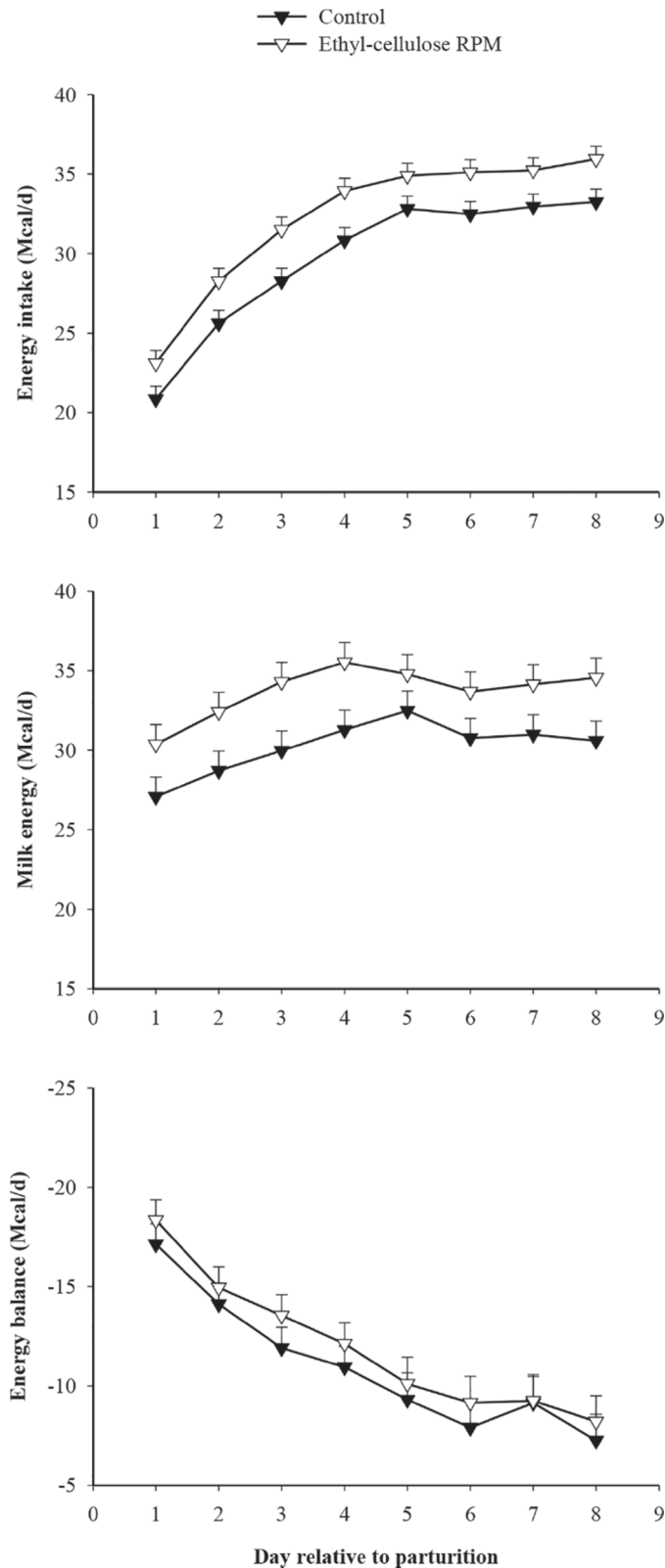
The NRC (2001) model assumes that the MP supply is first used to meet maintenance requirements, which are a function of BW (endogenous urinary and scurf proteins) and DMI (endogenous MP and metabolic fecal protein). The remainder is used for productive functions such as gestation, growth, and milk protein synthesis (Arriola Apelo et al., 2014). Corroborating results from previous studies using different sources of RPM, the MP balance was positive prepartum and negative postpartum regardless of treatment (Osorio et al., 2013). Considering that there was no difference in cow BW prepartum, it was expected that RPM-supplemented cows had a more positive MP balance

**Table 5.** Effects of ethyl-cellulose rumen-protected methionine (RPM) supplementation to multiparous Holstein dairy cows during the close-up (from -28 d to parturition), fresh (from 1 to 30 DIM), and high-producing (from 31 to 60 DIM) periods on net energy (NE) intake, milk energy, and energy balance

Item	Control	Ethyl-cellulose RPM	SEM	P-value		
				Treatment <sup>1</sup>	Time	Treatment × time
Fresh period						
NE intake	26.7	29.6	0.48	<0.01	<0.01	0.93
NE lactation	29.2	33.2	1.05	<0.01	<0.01	0.95
NE maintenance	10.3	10.5	0.24	0.57	<0.01	0.73
Energy balance	-13.5	-14.7	0.91	0.35	<0.01	0.92
High-producing period						
NE intake	32.9	35.3	0.36	<0.01	0.28	0.43
NE lactation	31.2	34.0	1.12	0.05	0.43	0.99
NE maintenance	10.3	10.5	0.24	0.57	<0.01	0.73
Energy balance	-6.41	-7.31	1.13	0.60	0.06	0.89

<sup>1</sup>Ethyl-cellulose RPM effect.





**Figure 3.** Effects of ethyl-cellulose rumen-protected methionine (RPM) supplementation to multiparous Holstein cows from -28 through 60 DIM on energy intake, milk energy, and energy balance. Error bars represent SEM.

prepartum. This positive MP balance during the prepartum period was not only from the RUP but also from positive RDP values in both treatments.

Despite the greater estimated supply of MP in ethyl-cellulose RPM-supplemented cows, according to NRC (2001), a more negative MP balance was observed postpartum in this group. This was likely due to the greater MP required to sustain increases in milk and milk protein yield with ethyl-cellulose RPM supplementation. Postpartum, the estimated increase of MP requirement for cows fed ethyl-cellulose RPM was 257 and 122 g/d for fresh and high-producing periods, respectively. The more negative protein balance in ethyl-cellulose RPM cows might have resulted in greater protein mobilization from muscle to match requirements. However, we did not observe differences in BW between groups throughout the study.

The duodenal supply of Lys and Met desired to maximize milk protein content and yield during established lactation has been suggested to be 7.2 and 2.4% of MP, respectively (NRC, 2001). In the present study, Lys was roughly constant at 6.5% of MP, whereas ethyl-cellulose RPM supplementation increased Met from 1.70 to 2.25% of MP on average. Also, we estimated that ethyl-cellulose RPM supplementation decreased the duodenal Lys:Met ratio from 3.78, 3.71, and 3.71 in the prepartum, fresh, and high-production periods, respectively, to 2.81:1, 2.88:1, and 2.82:1.

### DMI

Previous studies with RPM supplementation have resulted in inconsistent responses in DMI (Ordway et al., 2009; Chen et al., 2011; Osorio et al., 2013). For instance, Patton (2010) compiled data from 35 studies in which RPM was fed and reported a high heterogeneity in the DMI response, which indicated that other factors such as differences in level of RPM supplementation, length of feeding period, or stage of lactation likely confounded the results. In our study, ethyl-cellulose RPM supplementation increased DMI not only in the prepartum and immediate postpartum periods as previously observed (i.e., Osorio et al., 2013; Zhou et al., 2016b) but also when fed in the high-producing period up to 60 DIM.

The consistently greater DMI in RPM-fed cows during the transition period has been associated with better immunometabolic status, a reduction in inflammation and oxidative stress status, and better liver function (Osorio et al., 2014; Zhou et al., 2016a). The lower concentration of the enzyme GGT could be considered one indicator (Bertonni et al., 2008) of better liver function in ethyl-cellulose RPM-fed cows. Previously, Zhou et al. (2016a,b) demonstrated that cows with better liver

function had greater DMI. Hence, the positive effect of ethyl-cellulose RPM on liver function can explain, at least in part, the greater DMI.

In the context of feed intake regulation, the hepatic energy status has been proposed as a major regulator in dairy cows—that is, as hepatic oxidative fuel supply increases, the brain is signaled via the vagal afferent nerve to reduce intake (Allen et al., 2009). Although it is thought that propionate and fatty acids are primary fuels that cause hypophagia in ruminants (Allen et al., 2009), more recent data suggest that fatty acids are the most quantitatively important hepatic oxidative substrates during the periparturition period (McCarthy et al., 2015a,b). Thus, the tendency for a greater concentration of fatty acids in early lactation in the control group could have been associated with an increase in acetyl CoA oxidized within the tricarboxylic acid cycle (Drackley et al., 2001) and may have been one of the causes for the lower DMI compared with ethyl-cellulose RPM-fed cows.

### Milk Production and Milk Composition

Overall, RPM supplementation has resulted in inconsistent responses in milk yield (Ordway et al., 2009; Chen et al., 2011; Osorio et al., 2013). Previous studies did not detect increases in milk yield when RPM or Met analog was fed (Piepenbrink et al., 2004; Socha et al., 2005; Ordway et al., 2009; Preynat et al., 2009), whereas others observed positive responses when supplied until 30 DIM (Overton et al., 1996; Piepenbrink

et al., 2004; Osorio et al., 2013; Sun et al., 2016; Zhou et al., 2016b). To our knowledge, our study is the first to demonstrate that RPM supplementation elicits a sustained increase in milk production from the immediate postpartum period (+4.1 kg/d until 30 DIM) through the peak of lactation (+4.4 kg/d from 30 to 60 DIM). This increase in milk yield is likely related to the greater DMI in ethyl-cellulose RPM-supplemented cows.

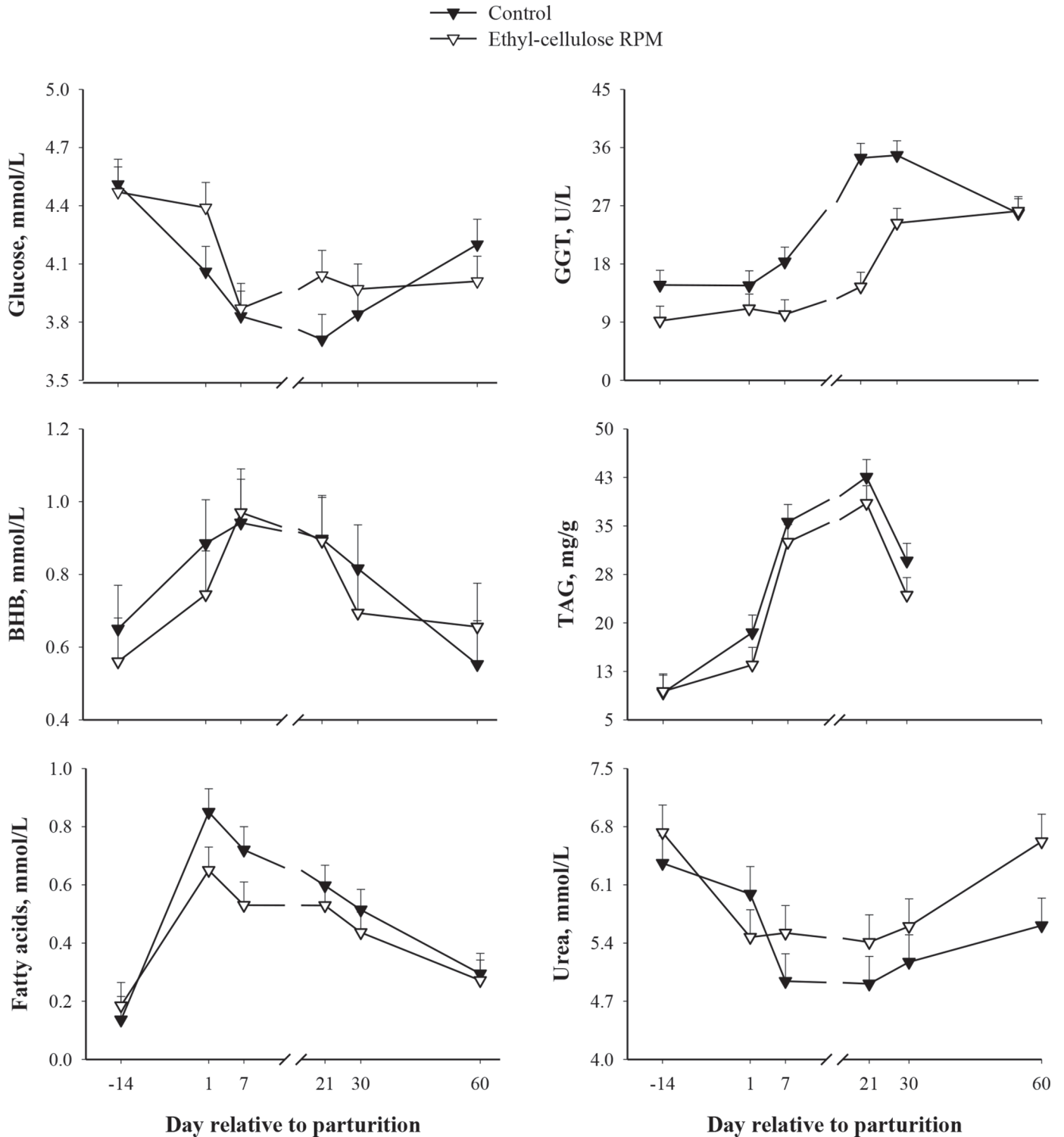
The increase in ECM from 4.3 to 4.7 kg/d with ethyl-cellulose RPM supplementation between the fresh and high-producing periods was associated with positive effects on the yield of milk components (i.e., milk protein content and yield), which underscores the fact that milk protein early postpartum is directly affected by the adequacy of AA in the MP (NRC, 2001). From a meta-analysis, Patton (2010) observed that cows that were Met deficient responded to RPM supplementation with greater milk protein and milk fat yield. The milk protein response in the present study was similar to previous results from our group (Osorio et al., 2013; Zhou et al., 2016b). It is also noteworthy that the milk protein yield responses detected during the fresh period in the present study and in previous studies from our laboratory (Osorio et al., 2013; Zhou et al., 2016b) were greater (from 120 to 190 g/d) than the values reported by Patton (2010).

Similar to our current findings, a previous study using RPM (Osorio et al., 2013) detected an increase in milk fat yield. An unexplored area of EAA metabolism in dairy cows is the potential effect of these nutrients

**Table 6.** Effects of ethyl-cellulose rumen-protected methionine (RPM) supplementation to multiparous Holstein dairy cows during the close-up (from -28 d to parturition), fresh (from 1 to 30 DIM), and high-producing (from 31 to 60 DIM) periods on blood biomarkers and liver triacylglycerol (TAG) concentrations

Item	Control	Ethyl-cellulose RPM	SEM	P-value			
				Treatment <sup>1</sup>	Time	Treatment × time	
<b>Blood</b>							
BHB, mmol/L	1.10	0.97	0.12	0.75	0.05	0.72	
Glucose, mmol/L	4.13	4.24	0.08	0.21	<0.01	0.45	
Fatty acids, mmol/L	0.71	0.63	0.05	0.08	<0.01	0.64	
Urea, mmol/L	5.73	5.89	0.33	0.28	<0.01	0.41	
γ-Glutamyl transferase, U/L	18.2	11.5	3.80	0.03	<0.01	0.38	
Nitric oxide, μmol/L	24.3	24.0	0.14	0.60	<0.01	0.52	
Nitrite, μmol/L	6.34	5.70	0.52	0.16	<0.01	0.62	
Nitrate, μmol/L	18.1	18.6	0.11	0.38	<0.01	0.29	
Calcium, mmol/L	2.44	2.48	0.02	0.19	<0.01	0.81	
Chlorine, mmol/L	105	104	0.30	0.31	<0.01	0.72	
Potassium, mmol/L	4.71	4.70	0.06	0.87	0.42	0.88	
Magnesium, mmol/L	0.94	0.96	0.02	0.36	<0.01	0.90	
Sodium, mmol/L	144	143	0.49	0.26	<0.01	0.72	
Phosphorus, mmol/L	2.14	2.15	0.04	0.96	<0.01	0.39	
Zinc, mmol/L	12.5	12.2	0.22	0.30	<0.01	0.56	
<b>Liver</b>							
TAG, mg/g of wet wt	31.1	29.3	2.71	0.38	<0.01	0.35	

<sup>1</sup>Ethyl-cellulose RPM effect.



**Figure 4.** Effects of ethyl-cellulose rumen-protected methionine (RPM) supplementation to multiparous Holstein cows from  $-28$  through  $60$  DIM on blood glucose, BHB, fatty acids, urea and  $\gamma$ -glutamyl transferase (GGT), and liver triacylglycerol (TAG; from  $-14$  to  $21$  DIM). Data for d  $30$  and  $60$  are included for comparison only. Error bars represent SEM.

on mRNA expression of lipogenic target genes. For instance, Li et al. (2016) using an in vitro model observed that an optimal AA balance stimulated the mRNA

expression of enzymes related to de novo FA synthesis (ACACA, FASN) and desaturation (SCD), TAG synthesis (LPIN1), and transcription regulation of lipid

synthesis (SREBF1, INSIG1) in bovine mammary cells. The effects of Met supplementation on regulation of mammary fatty acid synthesis deserve further investigation.

In our study, supplementation of ethyl-cellulose RPM increased energy intake but did not reduce negative energy balance compared with controls. This result was expected because we observed greater milk energy output in ethyl-cellulose RPM-supplemented cows due to the increase in milk yield and milk protein yield. A previous study (Gowanlock et al., 2015) reported a similar magnitude of negative energy balance when supplementing ethyl-cellulose RPM up to 30 DIM. In addition, the tendency for lower fatty acids and the lower GGT concentrations in ethyl-cellulose RPM-supplemented cows is indicative of an improvement in metabolic status, which has been associated with an increase in net energy efficiency due in part to a decrease in energy demands by the immune system (Trevisi et al., 2010; Kvidera et al., 2017).

### **Metabolism Biomarkers and Liver TAG**

Epidemiological data have established a positive association between increased blood concentrations of fatty acids and BHB and negative health and production outcomes at both the individual cow and herd levels (Duffield et al., 2009; Ospina et al., 2013). In our study, although we did not observe differences between treatments for BHB concentration, ethyl-cellulose RPM supplementation tended to reduce fatty acid concentration. A recent study also reported lower fatty acid concentrations from 1 to 21 DIM in cows supplemented with ethyl-cellulose RPM (Sun et al., 2016). Therefore, it is reasonable to speculate that ethyl-cellulose RPM-supplemented cows in the present study were able to metabolize circulating fatty acids more efficiently either in the liver or through uptake by the mammary gland or other peripheral tissues.

### **CONCLUSIONS**

Results indicate that feeding ethyl-cellulose RPM to achieve a Lys:Met ratio of 2.8:1 (0.09–0.10% of diet DM) during the periparturient period enhanced lactation performance from 1 to 60 DIM. The greater milk production was, at least in part, driven by the greater voluntary DMI and better liver function. To our knowledge, this is the first study reporting that ethyl-cellulose RPM supplementation starting from 4 wk prepartum improves performance not only in the immediate postpartum period but also through the peak of lactation.

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