Feeding rumen-protected lysine prepartum increases energy-corrected milk and milk component yields in Holstein cows during early lactation

L. K. Fehlberg,¹ A. R. Guadagnin,¹ B. L. Thomas,¹ Y. Sugimoto,² I. Shinzato,² and F. C. Cardoso^{1*}

¹Department of Animal Sciences, University of Illinois, Urbana 61801

²Ajinomoto Co. Inc., Tokyo, Japan 104

ABSTRACT

Feeding rumen-protected Lys (RPL) may be used to increase lactation performance in dairy cows; however, the effect of feeding RPL during the prepartum period and subsequent effect on postpartum performance is not well explored. Therefore, this experiment was conducted to determine the effects of feeding RPL (AjiPro-L Generation 3, Ajinomoto Heartland Inc., Chicago, IL) prepartum, postpartum, or both on performance, health, and blood metabolites. Seventy-five multiparous Holstein cows, blocked by parity, previous 305-d mature-equivalent milk production, expected calving date, and body condition score during the far-off dry period were assigned to 1 of 2 dietary treatments: total mixed ration with or without RPL in a randomized, complete block design. A 2 × 2 factorial arrangement of treatments was used. Prepartum (−28 d to calving), animals were fed a diet (forage, 68% of dietary DM) with RPL [PRE-L; 0.54% RPL of dietary dry matter intake (DMI)] or without RPL (control; PRE-C). After calving, half of the cows from each prepartum treatment group were assigned to a diet (forage, 55.5% of dietary DM) with RPL (PRE-L POST-L; PRE-C POST-L; 0.40% RPL of dietary DMI) or without RPL (PRE-C POST-C; PRE-L POST-C) until d 28 postpartum. Cows were milked twice a day and milk samples were taken on 7 ± 1.3, 14 ± 1.4, and 28 ± 1.1 d relative to calving (DRC). Milk yield and DMI were recorded daily. Blood samples were taken for plasma AA analysis on −7 ± 0.5, 0 ± 0.5, 7 ± 0.9, and 14 ± 0.9 DRC. Cows in PRE-L had greater body weight at −2 and −1 wk before calving compared with those in PRE-C, though body weight change from wk −4 to −1 was not different. Body weight (717 ± 6 kg) was greater and DMI (18.1 ± 0.7 kg) tended to be greater for cows in PRE-L POST-L and PRE-L POST-C compared with those that were in PRE-C

POST-L and PRE-C POST-C (707 ± 6 and 16.8 ± 0.7 kg, respectively). Energy-corrected milk (48.8 ± 1.9 kg/d), milk fat (1.9 ± 0.1 kg/d), milk true protein (1.4 ± 0.1 kg/d), milk casein (0.6 ± 0.04 kg/d), and milk lactose yields (2.1 ± 0.1 kg/d) were greater for cows in PRE-L POST-L and PRE-L POST-C compared with those that were in PRE-C POST-L and PRE-C POST-C (44.2 ± 1.9, 1.7 ± 0.1, 1.3 ± 0.1, 0.5 ± 0.04, 1.9 ± 0.1 kg/d, respectively). Plasma concentrations of Lys prepartum (69.8 ± 1.8 μM) increased for cows in PRE-L compared with those in PRE-C (62.5 ± 1.3 μM). In conclusion, RPL consumed prepartum tended to increase postpartum DMI and increased energy-corrected milk and milk component yields. This indicates that prepartum supply of intestinally available Lys is pertinent to postpartum performance. However, postpartum supply of intestinally available Lys had no effect on cows' performance.

Key words: amino acid, lysine, metabolizable protein, transition period

INTRODUCTION

The transition from gestation to lactation, also known as the periparturient period, is a critical time for dairy cows. This phase is typically defined as 3 wk before parturition through 3 wk after parturition (Drackley, 1999). Due to an increase in energy demands, most notably in the first week following parturition, it is almost impossible to avoid a negative energy balance, resulting in mobilization of body stores (Grummer, 1995). Therefore, the incidence of metabolic disorders increases dramatically (Drackley, 1999). There is also a negative protein balance due to an enhanced demand by the mammary tissues and conceptus growth, which is arguably of greater importance than a negative energy balance (Larsen et al., 2014). Impaired immune and tissue function and decreased proliferation of visceral and liver tissues may occur if duodenal flow of indispensable AA (IAA) is limited during the periparturient period (Connell et al., 1997; Li et al., 2007; Larsen et al., 2014). Notably, Met and Lys are often the most limit-

Received March 16, 2020.

Accepted July 23, 2020.

*Corresponding author: cardoso2@illinois.edu

ing AA in dairy cattle diets (NRC, 2001). Previously, the recommended amounts of intestinal supply of Lys and Met were 7.2 and 2.4% of total protein digested in the small intestine, respectively (NRC, 2001). However, expressing IAA requirements as a concentration of the diet can lead to deficiencies of these if DMI is not as high as predicted, which is common during the periparturient period (Vyas and Erdman, 2009). Due to this, amounts of IAA (g/d) is a more accurate unit of measurement. Bell et al. (2000) suggested increasing the amount of MP provided during the prepartum period to 1,000 g/d compared with the previous NRC (2001) recommendation of 742 g/d. However, it is important to note that MP amounts may vary depending on the equation used to calculate this value. For example, Bell et al. (2000) postulated that the NRC (2001) formula overestimates the efficiency of AA uptake by the uterus during the prepartum period, thereby underestimating the MP requirement. Inconsistencies in recommendations and expression of IAA content in the diet make it challenging to determine the actual requirement during the periparturient period for dairy cows (Chalupa and Sniffen, 1991).

Milk protein synthesis can be enhanced and mobilization of AA from tissues can be decreased by improving the duodenal flow of IAA (Carder and Weiss, 2017). Removal of Lys across hepatic tissue is limited; therefore, Lys is distributed to other tissues, such as skeletal muscle and the mammary gland (Lapierre et al., 2005). Feeding rumen-protected Lys (**RPL**) and rumen-protected Met (**RPM**) during the periparturient period has increased milk and milk protein yields of dairy cows (Xu et al., 1998; Socha et al., 2005; Osorio et al., 2013). It was suggested that the greatest response to intestinally supplied IAA is during early lactation, and likely this response occurs when IAA are fed prepartum (Overton et al., 1996; Socha et al., 2005). This was validated by a reduced lactational performance when RPL or RPL and RPM were consumed only postpartum compared with when they were consumed prepartum and postpartum, though the physiological mechanism supporting this response has not been verified (Wu et al., 1997; Socha et al., 2005). However, study design with continuous feeding of RPL and RPM throughout the periparturient period make it difficult to decipher prepartum and postpartum effects separately or the effect of prepartum supply on postpartum performance.

Though the need for intestinally available Lys in lactating cows has been verified (NRC, 2001), the requirement of intestinally available Lys of the transitioning dairy cow has not been totally explored. Though Lys is present in feedstuffs, Lys is often limiting, and variable amounts will reach the intestine for direct supply to the cow. For this reason, RPL is a more consistent

means to deliver Lys to the intestine (Chalupa and Sniffen, 1991). Feeding RPL can be used to increase lactation performance in dairy cows; however, the effect of feeding RPL during the prepartum and postpartum periods, independently, on cows' performance is not well explored. Therefore, the objective of this study was to determine the effects of feeding a commercially available RPL prepartum, postpartum, or both in diets of multiparous Holstein cows on their production parameters, health, and plasma AA concentrations. We postulated that feeding RPL during the periparturient period would positively affect production during the prepartum and postpartum periods by balancing for AA.

MATERIALS AND METHODS

Animal Care and Housing

All experimental procedures were approved by the University of Illinois (Urbana-Champaign) Institutional Animal Care and Use Committee (#18157). The experimental period was from September 2018 to July 2019. Cows were dried off at -57 ± 21 d relative to expected calving (**DREC**) and consumed a common straw-based diet until -30 DREC. At -30 DREC, dry cows were moved to an enclosed ventilated barn with access to sand-bedded freestalls and were fed a common prepartum diet for 2 d to allow for adaptation to the new environment and diet. The experimental period began at -28 DREC. Dry cows were fed a basal prepartum diet once daily at approximately 0530 h using an individual feeding system (American Calan Inc., Northwood, NH). When calving was imminent, cows were moved to a stall bedded with straw until calving. After calving, cows were housed in a tiestall barn until 28 d relative to calving (**DRC**) and fed a basal postpartum diet once daily at 0630 h. Daily DMI was determined for each cow by weighing refusals and total amount fed and determining the difference on a DM basis. Cows were fed for 10% refusals to allow for ad libitum feed intake. All cows had free access to water. After 28 DRC, cows were returned to the farm herd. Diets (TMR) were formulated using AMTS.Cattle.Pro version 4.7 (2017, AMTS LLC, Groton, NY) to meet or exceed recommendations (Table 1). The prepartum diet was formulated for dry cows at 694 kg of BW and a predicted DMI of 13 kg/d. The postpartum diet was formulated for cows at 14 DIM, 733 kg of BW, producing 39 kg of milk/d with a target of 3.7% milk fat and 3.2% milk protein, and a predicted DMI of 19 kg/d. Temperature and humidity were monitored at 5-min intervals for the prepartum and postpartum barns by using HOBO Pro logger (Onset Computer Corp.,

Pocasset, MA). Temperature-humidity index was calculated using the equation of McDowell et al. (1979).

Experimental Design and Dietary Treatments

A total of 89 multiparous pregnant Holstein cows started the study [BW (mean $\pm$ SD) = 791 $\pm$ 84 kg] and were blocked by parity (3.3 $\pm$ 1.1), previous 305-d mature-equivalent milk production (11,363 $\pm$ 1,860 kg), expected calving date, and BCS during the far-off dry period (3.76 $\pm$ 0.84) and were assigned to 1 of 2 dietary treatments: TMR with RPL (AjiPro-L Gen-

eration 3, Ajinomoto Heartland Inc., Chicago, IL) or without in a randomized, complete block design. A 2 $\times$ 2 factorial arrangement of treatments was used. Prepartum (–28 d to calving), animals were fed a diet with RPL (**PRE-L** (n = 43); 0.54% RPL of dietary DMI) or without RPL [control; **PRE-C** (n = 40)] top dressed in a carrier of 300 g of dried sugarcane molasses. After calving, half of the cows from each prepartum treatment group were assigned to a diet with RPL [**PRE-L POST-L** (n = 18); **PRE-C POST-L** (n = 20); 0.40% RPL of dietary DMI] or a diet without RPL [**PRE-C POST-C** (n = 18); **PRE-L POST-C** (n = 19)] until d 28 postpartum. According to the manufacturer, there is an 80% rumen bypass and 80% intestinal digestibility of this encapsulated RPL product. This would provide 1.4 g of intestinally available Lys prepartum and 1.0 g of intestinally available Lys postpartum per kg of DMI. Top dress was applied to the TMR once daily following the morning feeding. A total data set of 75 cows concluded the study, resulting in 19 blocks after accounting for exclusions. All blocks contained 1 cow from each treatment, resulting in 4 cows per block, except block 19 contained 6 cows with at least 1 cow from each treatment (an extra cow from PRE-L POST-C and PRE-L POST-L were added). Cows were automatically excluded if they calved with twins or if they did not consume the treatment for at least 16 d during the prepartum period. Of cows included in the final data set, β -casein genetics were determined and supplied in Supplemental Table S1, <https://doi.org/10.3168/jds.2020-18542>.

According to AMTS.Cattle.Pro prediction, cows in PRE-C consuming the prepartum diet received 1.17 kg/d of MP, resulting in 6.86% MP as Lys, 2.98% MP as Met, and 2.71% MP as His with a Lys:Met of 2.30 and His:Met of 0.92; whereas cows in PRE-L received 1.19 kg/d of MP, resulting in 8.24% MP as Lys, 2.94% MP as Met, and 2.67% MP as His with a Lys:Met of 2.80 and His:Met of 0.92. Cows in PRE-L POST-C and PRE-C POST-C consuming the postpartum diet received 2.28 kg/d of MP, resulting in 6.27% MP as Lys, 2.54% MP as Met, and 2.42% MP as His with a Lys:Met of 2.46 and His:Met of 0.94; while cows in PRE-L POST-L and PRE-C POST-L received 2.22 kg/d of MP, resulting in 7.15% MP as Lys, 2.55% MP as Met, and 2.40% MP as His with a Lys:Met of 2.80 and His:Met of 0.93. Samples of TMR for prepartum and postpartum diets were analyzed weekly for DM by oven drying at 110°C (AOAC International, 1995a).

Data Collection and Sampling Procedures

Samples of TMR were collected weekly, stored at –20°C, and composited monthly. Composited samples

Table 1. Ingredient composition of prepartum and postpartum diets fed to all cows during the experimental period

Ingredient ¹	Prepartum ²	Postpartum ³
Corn silage ⁴	31.06	39.38
Canola meal	1.45	5.36
Alfalfa hay	—	20.95
Wheat middlings	4.10	—
Corn gluten feed	6.69	—
Soybean meal, 48% CP	2.19	—
Wheat straw	40.25	—
Dry ground corn grain	0.16	15.26
Rumen-protected methionine ⁵	0.12	0.09
Rumen-inert fat ⁶	—	1.93
Expeller soybean meal ⁷	5.74	6.66
Anionic mineral supplement ⁸	3.85	—
Urea 46%	0.23	0.30
Magnesium oxide	—	0.09
Magnesium sulfate	0.25	—
Dicalcium phosphate	—	0.33
Molasses beet	—	4.43
Calcium carbonate	2.08	—
Vitamin and mineral mix prepartum ⁹	1.31	—
Vitamin and mineral mix postpartum ¹⁰	—	4.73

¹Ingredients expressed as percentage of DM.

²A prepartum diet fed an estimated 28 d before calving until parturition.

³A postpartum diet fed 0 to 28 d after calving.

⁴Corn silage averaged 34.7% of DM across diets.

⁵Smartamine M (80% bioavailability providing 1.05 g of Smartamine M/kg of DMI; Adisseo, Alpharetta, GA).

⁶Energy booster 100 (Milk Specialties Global, Eden Prairie, MN).

⁷Soy Plus (Landus Cooperative, Ames, IA).

⁸Animate (Phibro Animal Health Corporation, Teaneck, NJ).

⁹Vitamin and mineral mix prepartum was formulated to contain 28.19% Ca, 3.99% Na, 5.90% Cl, 6.11% Mg, 0.02% K, 0.22% S, 3.70 mg/kg Co, 510.61 mg/kg Cu, 97.47 mg/kg I, 1,257.27 mg/kg Fe, 2,424.62 mg/kg Mn, 27.68 mg/kg Se, 27.68 mg/kg organic Se, 1,000.74 mg/kg Zn, 720.12 kIU/kg vitamin A, 219.00 kIU/kg vitamin D₃, 7,125.16 IU/kg vitamin E, and 1.90 g/kg monensin (Rumensin, Elanco, Greenfield, IN).

¹⁰Vitamin and mineral mix postpartum was formulated to contain 12.51% Ca, 14.06% Na, 9.60% Cl, 3.18% Mg, 6.48% K, 0.19% S, 26.93 mg/kg Co, 301.01 mg/kg Cu, 40.22 mg/kg I, 678.25 mg/kg Fe, 1,519.35 mg/kg Mn, 8.62 mg/kg Se, 4.47 mg/kg organic Se, 1,621.05 mg/kg Zn, 43.34 kIU/kg vitamin A, 10.89 kIU/kg vitamin D₃, 466.41 IU/kg vitamin E, 4.23 mg/kg biotin, 46.65 mg/kg thiamine, and 0.35 g/kg monensin (Rumensin, Elanco).

were analyzed for DM, CP, ADF, NDF, lignin, starch, fat, ash, Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, and S using wet chemistry methods (Dairy One Cooperative Inc., Ithaca, NY; Table 2). The physical characteristic of the TMR, based on the Penn State Particle Separator (Kononoff et al., 2003), was performed weekly using upper (19-mm pore size), middle (8-mm pore size), lower (4-mm pore size) sieves, and the pan.

Cows were milked at approximately 0600 and 1600 h. Milk weights were recorded at each milking. Milk samples were collected at both 0600 and 1600 h milkings at 7 ± 1.3 (mean $\pm$ SD), 14 ± 1.4 , and 28 ± 1.1 DRC, with a minimum and maximum of ± 2 DRC for all collection times. A preservative (800 Broad Spectrum Microtabs II; D&F Control Systems, Inc., San Ramon, CA) was added to samples, which were then refrigerated and composited weekly in proportion to daily milk yield. Composited milk samples were sent to a commercial laboratory (Dairy One Cooperative Inc.) to be analyzed for contents of fat, true protein, casein, MUN, lactose, total solids, and SCC using mid-infrared procedures (AOAC International, 1995b). A subsample of milk samples (PRE-L POST-L, $n = 11$; PRE-L POST-C, $n = 13$; PRE-C POST-L, $n = 13$; PRE-C POST-C, $n = 12$; for each collection time point) were also sent to a commercial laboratory (ADM Laboratories, Clovis, NM) for concentrations of preformed (C18 and longer), de novo (C4 to C15), and mixed fatty acid (FA; C16:0, C16:1, and C17:0) analyses (Wojciechowski and Barbano, 2016).

Colostrum was obtained from each cow at the first milking following calving (3.83 ± 3.63 h after calving). Colostrum weight, Brix (MISCO PA202x-400 Digital Refractometer, Solon, OH) and IgG concentration (Colostrometer, Biogenics, Florence, OR; Bartier et al., 2015) were recorded immediately following collection. Colostrum samples were sent to a commercial laboratory (Dairy One Cooperative Inc.) to be analyzed for content of fat by ether extraction (AOAC International, 1995c), true protein by Kjeldahl (AOAC International, 1995d), and total solids by convection (AOAC International, 1995e).

Beta-hydroxybutyrate from whole blood was analyzed on 7 ± 0.71 and 14 ± 0.57 DRC immediately after sampling using a digital cow-side ketone monitor (Nova Max Plus, Nova Biomedical Corporation, Waltham, MA). Plasma concentrations of AA were measured on -26 ± 4.6 , -7 ± 0.5 , 0 ± 0.5 , 7 ± 0.9 and 14 ± 0.9 DRC using an ultra-performance liquid chromatography–MS (Waters, Milford, MA; Armenta et al., 2010) with the derivatization method (AccQ-Tag Derivatization) provided by the manufacturer. There was a minimum and maximum of ± 2 DRC for all col-

Table 2. Mean chemical composition ($\pm$ SD) for prepartum and postpartum diets fed to all cows throughout the experimental period¹

Item	Prepartum ²	Postpartum ³
DM, %	43.4 $\pm$ 1.42	45.7 $\pm$ 1.64
CP, % of DM	14.2 $\pm$ 0.68	16.8 $\pm$ 1.06
ADF, % of DM	28.4 $\pm$ 2.80	20.9 $\pm$ 1.77
NDF, % of DM	44.8 $\pm$ 2.75	31.3 $\pm$ 3.29
Lignin, % of DM	4.44 $\pm$ 0.74	3.80 $\pm$ 0.49
Starch, % of DM	14.0 $\pm$ 1.69	24.4 $\pm$ 2.62
Crude fat, % of DM	3.03 $\pm$ 0.21	4.95 $\pm$ 0.51
Ash, % of DM	10.3 $\pm$ 1.34	9.16 $\pm$ 0.74
NE _L , Mcal/kg of DM ⁴	1.44 $\pm$ 0.03	1.67 $\pm$ 0.05
Ca, % of DM	1.46 $\pm$ 0.35	1.12 $\pm$ 0.21
P, % of DM	0.37 $\pm$ 0.04	0.41 $\pm$ 0.04
Mg, % of DM	0.50 $\pm$ 0.07	0.38 $\pm$ 0.03
K, % of DM	1.12 $\pm$ 0.11	1.75 $\pm$ 0.17
Na, % of DM	0.17 $\pm$ 0.04	0.63 $\pm$ 0.07
S, % of DM	0.45 $\pm$ 0.04	0.26 $\pm$ 0.03
Fe, mg/kg	547 $\pm$ 205	351 $\pm$ 94
Zn, mg/kg	60.2 $\pm$ 13.0	113 $\pm$ 20.7
Cu, mg/kg	14.2 $\pm$ 2.11	16.5 $\pm$ 1.84
Mn, mg/kg	91.9 $\pm$ 17.5	99.3 $\pm$ 13.7

¹Mean diet composition sampled once weekly and composited monthly throughout experimental period ($n = 10$); diets were formulated using AMTS.Cattle.Pro Version 4.7 (Groton, NY).

²The prepartum diet was fed from an estimated -28 d before calving until parturition ($\pm$ SD).

³The postpartum diet was fed from 0 to 28 d after calving ($\pm$ SD).

⁴Diet NE_L were estimated using NRC (2001).

lection time points for BHB and plasma concentrations of AA.

Prepartum and postpartum energy balance (**EBAL**) were calculated weekly with the following equation (NRC, 2001):

$$\begin{aligned} \text{EBAL prepartum} &= \text{energy intake} \\ &(\text{Mcal of NE}_L/\text{d}) - [\text{maintenance requirement} \\ &(\text{Mcal of NE}_L/\text{d}) + \text{pregnancy requirement} \\ &(\text{Mcal of NE}_L/\text{d})], \end{aligned}$$

$$\begin{aligned} \text{EBAL postpartum} &= \text{energy intake} \\ &(\text{Mcal of NE}_L/\text{d}) - [\text{maintenance requirement} \\ &(\text{Mcal of NE}_L/\text{d}) + \text{lactation requirement} \\ &(\text{Mcal of NE}_L/\text{d})]. \end{aligned}$$

Health Events

Health disorders included retained placenta, dystocia, milk fever, displaced abomasum, clinical mastitis, and subclinical ketosis. Retained placenta was defined as a placenta that failed to deliver completely more than 12 h after calf delivery; dystocia was defined based on the degree of calving difficulty (0 = calved by herself;

1 = 1 person was needed to assist; 2 = 2 people were needed to assist; 3 = cesarean section); milk fever and displaced abomasum were diagnosed by a veterinarian; clinical mastitis was diagnosed by altered milk composition confirmed by positive microbiological culture; and subclinical ketosis was diagnosed using a blood ketone meter (Nova Max, Nova Diabetes Care, Billerica, MA) when $1.2 \text{ mmol/L} \leq \text{BHB} \leq 3.0 \text{ mmol/L}$.

Health evaluations and visual assessments were performed weekly. Rectal temperature was measured using a GLA M700 Thermometer (GLA Agricultural Electronics, San Luis Obispo, CA). Heart rate of cows was measured by palpating the femoral artery for 15 s and multiplying this number by 4. Respiration rate of cows was obtained via visual observation of breathes taken in a 30 s period and multiplied by 2. General appearance was scored using a similar method to Krause et al. (2009): using a scoring system 1 through 4, where 4 = bright and alert; 1 = down cow, unable to get up. Fecal scores on a 1 through 4 scale were allocated according to Krause et al. (2009): 1 = runny, liquid consistency; 4 = hard, dry appearance, original form not distorted on impact and settling. Body weight was measured (Ohaus digital scale, model CW-11, Newark, NJ) and BCS was assigned in quarter-unit increments for each cow weekly (Ferguson et al., 1994). Three people independently assigned a BCS score and the average score was used for statistical analysis.

Lameness score was measured once a week based on an ordinal scale from 1 to 5, where 1 = not lame, walks and stands normally with a level back; 2 = stands with a flat back, but arches when walks; 3 = stands and walks with an arched back and short strides with one or more legs; 4 = arched back standing and walking, favoring one or more limbs but can still bear some weight on them; and 5 = pronounced arching of back, reluctant to move, with almost complete weight transfer off the affected limb (Sprecher et al., 1997).

Statistical Analyses

The prepartum period was restricted to -28 DREC through calving and the postpartum period was restricted to 1 through 28 DRC. Daily prepartum and postpartum DMI and milk yield were condensed to weekly averages. Prepartum and postpartum data were analyzed separately. Prior to consumption of experimental treatments, initial prepartum BCS, BW, and DMI, were recorded and used as a covariate for prepartum data and initial prepartum plasma AA were analyzed as a covariate for prepartum and postpartum data. The prepartum period for plasma AA consisted of -7 DRC and 0 DRC collection times and the post-

partum period used 7 and 14 DRC. Statistical analyses were conducted using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). The model for prepartum had the inclusion of RPL prepartum (PRE) or not, time [T; wk or DRC], and PRE $\times$ T interaction. The model for postpartum contained the inclusion of PRE or not, inclusion of RPL postpartum (POST), T, PRE $\times$ T interaction, POST $\times$ T interaction, PRE $\times$ POST interaction, and PRE $\times$ POST $\times$ T interaction. The following model was used:

$$Y_{jklm} = \mu + A_j + B_k + T_l + (AT)_{jl} + (BT)_{kl} + (AB)_{jk} + (ABT)_{jkl} + C_m + \varepsilon_{jklm},$$

where Y_{jklm} = the observations for dependent variables; μ = the overall mean; A_j = the fixed effect of PRE; B_k = the fixed effect of POST; T_l = the repeated effect of T; $(AT)_{jl}$ = the interaction of PRE and T; $(BT)_{kl}$ = the interaction of POST and T; $(AB)_{jk}$ = the interaction of PRE and POST; $(ABT)_{jkl}$ = the interaction of PRE and POST and T; C_m = the random effect of cow; and ε_{jklm} = the random residual error. Cow was used as the experimental unit. Denominator degrees of freedom was estimated using Kenward-Roger method (Littell, 2002). Distribution of the residuals was evaluated to determine normality and homoscedasticity. A Q-Q plot and box plot were used to determine outliers (calculated by multiplying the interquartile range by 3 and subtracting or adding from the corresponding quartile). A log-transformation was used for the variables milk SCC, colostrum fat, colostrum true protein, and colostrum IgG concentration to enhance homogeneity of the distribution of residuals. Least squares means were back transformed for these variables. Frequency analysis was performed using the FREQ procedure of SAS considering the binary response variable of retained placenta, dystocia, milk fever, displaced abomasum, clinical mastitis, and subclinical ketosis using the chi-squared probability. Statistical significance for all analyses was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Diet Composition, Barn Characteristics, and Cow Exclusions

The ingredient composition of the diets is in Table 1. Analyzed nutrients from the experimental diets are in Table 2. The physical characteristics of the prepartum TMR were (mean $\pm$ SD) $6.1 \pm 3.0\%$ on upper, $47.8 \pm 5.3\%$ on middle, $20.0 \pm 3.0\%$ on lower sieves, and $26.1 \pm 6.7\%$ in the pan, and postpartum TMR were $5.2 \pm 1.8\%$ on upper, $38.8 \pm 5.0\%$ on middle, $20.7 \pm 4.8\%$ on

lower sieves, and $35.3 \pm 7.3\%$ in the pan. The average temperature-humidity index was 44 ± 12 for the prepartum barn and 51 ± 7 for the postpartum barn. Four cows were excluded due to twins (PRE-C POST-C, $n = 1$; PRE-C POST-L, $n = 1$, PRE-L POST-C, $n = 1$; PRE-L POST-L, $n = 1$) and 1 cow was excluded for calving too early (PRE-L POST-C, $n = 1$). One cow was excluded prepartum due to vaginal paralysis (treatment = PRE-C POST-L). The pre and postpartum data were excluded from 1 cow due to digestive problems prepartum and postpartum (PRE-L POST-L). Seven cows were excluded postpartum due to fracture (PRE-L POST-C, PRE-C POST-L, and PRE-L POST-L), leucosis (PRE-L POST-L), uterine prolapse (PRE-L POST-L), or digestive problems (PRE-L POST-C, PRE-L POST-L, and PRE-C POST-C); however, prepartum data for these cows were retained.

Intake, BW, and BCS Prepartum

Dry matter intake as a percentage of BW, EBAL, and BCS were not different between cows in PRE-L and those in PRE-C ($P > 0.80$; Table 3). As expected, there was an effect of week on DMI, DMI as a percentage of BW, BW, EBAL, and BCS during the prepartum period in which wk -1 relative to calving was less compared with the previous weeks ($P < 0.05$). There was no difference in BW comparing cows in PRE-L (808 kg) and those in PRE-C (803 kg; $P = 0.12$) and no difference in the BW change from wk -4 to -1 ($P > 0.10$). However, there was a PRE $\times$ wk interaction in which cows in PRE-L were heavier during wk -2 and -1 relative to calving compared with cows that were in PRE-C ($P = 0.05$; Figure 1).

Figure 1. Least squares means ($\pm$ SEM) for BW for 4 wk prepartum by inclusion of rumen-protected Lys (RPL) before calving (PRE). Cows were assigned to 1 of 2 dietary treatments (TMR with or without RPL) in a randomized complete block design. A 2×2 factorial arrangement of treatments was used. Prepartum (-28 d to calving), animals were fed a diet with RPL (PRE-L; 0.54% RPL of dietary DMI) or without RPL (control; PRE-C). After calving, half of the cows from each prepartum treatment group were assigned to a diet with RPL (PRE-L POST-L; PRE-C POST-L; 0.40% RPL of dietary DMI) or a diet without RPL (PRE-C POST-C; PRE-L POST-C) until d 28. Interaction of PRE $\times$ week: $P = 0.05$. * indicates a difference between treatments at that time point at $P = 0.03$.

Intake, BW, and BCS, Postpartum, and Lactation Performance

Postpartum production performance data are in Table 4. There was a tendency for cows in PRE-L to have greater DMI postpartum (18.1 kg) compared with those in PRE-C (16.8 kg; $P = 0.08$). There was no effect of PRE ($P > 0.37$) or POST ($P > 0.47$) on EBAL or milk yield. As expected, energy balance increased as wk increased ($P < 0.01$). Cows in PRE-L had greater

Table 3. Least squares means and associated SEM for prepartum DMI, energy balance (EBAL), BW, and BCS of Holstein cows consuming diets with rumen-protected Lys (RPL) or without (control) for 4 wk before calving

Prepartum variable ¹	Treatment ²			P-value ⁴	
	PRE-L	PRE-C	SEM ³	PRE	PRE $\times$ wk
DMI, kg/d	12.1	11.8	0.21	0.31	0.80
DMI, % of BW	1.46	1.39	0.05	0.33	0.60
EBAL, Mcal/d	1.92	1.18	0.44	0.23	0.76
BW, kg	808	803	2	0.12	0.05
BW change, (wk -4 to -1), kg	-22.0	-31.7	4.70	0.29	—
BCS	3.81	3.79	0.02	0.49	0.28

¹Data were collected daily or once per week and consolidated to weekly averages (DMI and EBAL) for 4 wk prepartum; EBAL was calculated based on NRC (2001).

²Dietary treatments consisted of a top dress either with RPL (PRE-L) or without (control; PRE-C) consumed for 4 wk before calving in a carrier of 300 g of dried molasses.

³Greatest value for SEM within treatment.

⁴Main effect of RPL prepartum (PRE) and wk and their interaction. There was an effect of wk for all variables and therefore was not included in the table.

BW postpartum (717 kg) compared with cows in PRE-C (706 kg; $P = 0.05$); however, there was no effect of POST on BW ($P = 0.15$). There was a POST $\times$ wk interaction in which cows in POST-L had a greater BCS than those in POST-C (Supplemental Figure S1, <https://doi.org/10.3168/jds.2020-18542>; $P = 0.04$). There was a PRE $\times$ wk and PRE $\times$ POST interaction for BHB in whole blood ($P < 0.05$), in which cows in PRE-L had lower BHB (0.59 mmol/L) concentrations in whole blood at 7 DRC compared with PRE-C (0.83 mmol/L), with no difference by 14 DRC.

There was greater ECM yield (48.8 kg/d) for cows in PRE-L compared with those in PRE-C (44.2 kg/d; $P < 0.05$). Milk fat, protein, casein, and lactose yield were greater for cows in PRE-L compared with those in PRE-C ($P < 0.05$). There was a tendency for a PRE $\times$ POST $\times$ wk interaction for milk fat concentration ($P = 0.09$), in which cows in PRE-C POST-L had greater milk fat concentration at wk 4 (4.56%) compared with those in PRE-C POST-C and PRE-L POST-L (3.78 and 3.80%, respectively; $P < 0.05$) and a tendency compared with those in PRE-L POST-C (4.02%; $P = 0.08$). Mixed FA and preformed FA as a percentage of fat tended to be greater for cow in PRE-L compared with those in PRE-C ($P = 0.07$) and a PRE $\times$ POST ($P = 0.09$) interaction for preformed FA as a percentage of fat. Cows in PRE-L POST-L had less preformed FA (37.8%) when compared with cows in PRE-L POST-C, PRE-C POST-L, and PRE-C POST-C (42.1, 43.6, and 42.3%, respectively). There were no treatment differences in de novo FA as a percentage of FA ($P > 0.25$). A tendency for a PRE $\times$ POST $\times$ wk interaction for SCC ($P = 0.08$) was demonstrated by greater SCC in cows in PRE-L POST-L at wk 1 compared with all other treatments; however, there were no differences by wk 4 ($P > 0.10$). There was a PRE $\times$ POST $\times$ wk interaction for milk yield/DMI in which cows in PRE-C POST-C (3.34) had greater efficiency than cows in PRE-C POST-L, PRE-L POST-C, and PRE-L POST-L at wk 2 after calving (2.53, 2.52, 2.43, respectively; $P < 0.05$) but no differences at wk 1, 3, or 4. Treatment effects on colostrum are in Supplemental Table S2, <https://doi.org/10.3168/jds.2020-18542>. There were no differences in colostrum yield, IgG concentration, fat, protein, and solids ($P > 0.10$).

Plasma AA and Metabolites, Health, Lameness, and Fecal Score

Plasma concentrations of AA and metabolites during the prepartum period are in Table 5. During the prepartum period, plasma concentration of Lys was greater for cows in PRE-L (69.8 μ M) compared with cows in PRE-C (62.5 μ M; $P = 0.009$). There was also a PRE $\times$

DRC interaction in which plasma concentration of Lys was greater for cows in PRE-L compared with cows in PRE-C on -7 DRC ($P = 0.002$) but there was no difference on 0 DRC. There was a PRE $\times$ DRC interaction for plasma concentration of Lys as a percentage of total AA and a percentage of IAA in which cows in PRE-L had greater concentrations at -7 and 0 DRC compared with cows in PRE-C ($P < 0.01$). Plasma concentrations of Met tended to decrease ($P = 0.10$) for cows in PRE-L compared with cows in PRE-C. Due to decreases in IAA, dispensable AA (DAA), and metabolites, plasma concentrations of total AA, total DAA, total branched-chain AA (BCAA), and total sulfur AA decreased ($P \leq 0.05$) and total IAA tended to decrease ($P = 0.08$) for cows in PRE-L compared with cows in PRE-C. There was an effect of DRC on all IAA, DAA (excluding Gln), metabolites, total AA, total IAA, total branched-chain AA, and total urea cycle AA ($P \leq 0.05$).

Plasma concentrations of AA and metabolites during the postpartum period are in Table 6. Plasma concentration of Lys as a percentage of IAA was greater for cows in POST-L (10.5%) compared with cows in POST-C (9.65%) resulting in an effect of POST ($P < 0.01$); however, there were no effects of PRE or POST on plasma concentrations of Lys and Lys as a percentage of total AA ($P > 0.19$). Plasma concentrations of Met were lesser for cows in PRE-L POST-L and PRE-L POST-C compared with those in PRE-C POST-L and PRE-C POST-C, resulting in an effect of PRE ($P = 0.02$). There was a tendency for plasma concentrations of total sulfur AA to be lesser for cows in PRE-L POST-L and PRE-L POST-C (92.2 and 93.2 μ M, respectively) compared with cows in PRE-C POST-L and PRE-C POST-C (96.7 and 101 μ M, respectively), resulting in a tendency for an effect of PRE ($P = 0.06$). There were no effects of PRE or POST for plasma concentrations of total AA, total IAA, total DAA, total BCAA, and total urea cycle AA postpartum ($P > 0.18$).

There was no effect of treatment on incidence of health disorders ($P > 0.10$). Treatments had no effect on mean fecal score prepartum ($P = 0.69$, overall LSM $\pm$ SE; 2.9 ± 0.10) nor postpartum ($P = 0.36$; overall LSM $\pm$ SE; 2.8 ± 0.12) and had no effect on lameness score prepartum ($P = 0.11$ overall LSM $\pm$ SE; 1.1 ± 0.06) nor postpartum ($P = 0.97$; overall LSM $\pm$ SE; 1.1 ± 0.09).

DISCUSSION

We hypothesized that cows receiving RPL during the transition period would have improved performance prepartum and postpartum due to balancing of Lys, which is of specific importance during the periparturient period, compared with those that did not.

Table 4. Least squares means and SEM for postpartum BW, BCS, BHB, and production parameters of Holstein cows fed a common lactation diet with rumen-protected Lys (RPL) or without (control) for 4 wk before calving and first 4 wk postpartum

Variable ¹	Treatment ²				P-value ⁴			
	PRE-L POST-L	PRE-L POST-C	PRE-C POST-L	PRE-C POST-C	SEM ³	PRE × wk	POST × wk	PRE × POST × wk
DMI, kg/d	18.4	17.8	17.4	16.3	0.74	0.08	0.22	0.71
DMI by BW	2.59	2.57	2.37	2.40	0.13	0.12	0.99	0.34
EBAL, Mcal/d ⁵	-14.6	-14.9	-14.7	-12.2	1.53	0.37	0.47	0.73
BW, kg	719	715	713	700	6	0.05	0.15	0.56
BW change (wk 1 to 4), kg	-28.8	-34.3	-33.0	-26.1	7.18	0.71	0.65	—
BCS	3.34	3.38	3.44	3.35	0.05	0.58	0.71	0.24
BHB, mmol/L ⁶	0.51 ^a	0.72 ^{ab}	0.76 ^b	0.70 ^{ab}	0.07	0.09	0.29	0.05
Milk yield								
Milk yield, kg/d	40.8	41.1	40.1	37.1	1.65	0.15	0.40	0.32
ECM, kg/d	49.0	48.8	46.7	41.7	1.91	0.02	0.15	0.23
Milk change (wk 1 to 4), %	152	149	144	149	5	0.43	0.88	0.43
Milk composition ⁷								
Fat, %	4.44	4.56	4.59	4.31	0.16	0.73	0.59	0.34
Fat, kg/d	1.91	1.94	1.87	1.61	0.09	0.04	0.19	0.37
De novo FA, % of fat	20.3	18.9	18.6	18.9	0.79	0.26	0.48	0.47
Preformed FA, % of fat	37.8 ^a	42.1 ^{ab}	43.6 ^b	42.3 ^{ab}	1.77	0.07	0.36	0.55
Mixed FA, % of fat	41.9	39.0	38.0	38.7	1.24	0.07	0.36	0.92
Protein, %	3.31	3.24	3.32	3.32	0.05	0.44	0.50	0.84
Protein, kg/d	1.43	1.38	1.34	1.25	0.05	0.04	0.18	0.47
Casein, %	2.70	2.65	2.71	2.71	0.05	0.47	0.52	0.35
Casein, kg/d	0.65	0.62	0.57	0.50	0.04	0.03	0.20	0.30
Lactose, %	4.77	4.74	4.73	4.78	0.04	0.99	0.70	0.74
Lactose, kg/d	2.09	2.04	1.93	1.83	0.08	0.02	0.34	0.33
MUN, mg/dL	12.2	12.1	11.6	11.9	0.53	0.48	0.82	0.73
SCC, × 1,000/mL	332	124	185	218	112	0.58	0.29	0.60
Milk/DMI	2.37	2.44	2.51	2.67	0.14	0.16	0.38	0.02

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).¹Data were collected daily or once per week and consolidated to weekly means (DMI and milk yield) for 4 wk postpartum.²Dietary treatments included at top dress with RPL prepartum and postpartum (PRE-L POST-L), with RPL prepartum and without RPL postpartum (PRE-L POST-C), without RPL prepartum and with RPL postpartum (PRE-C POST-L), and without RPL prepartum and postpartum (control; PRE-C POST-C) in a carrier of 300 g of dried molasses.³Greatest value of SEM within treatment.⁴Consists of the main effect of RPL prepartum (PRE), the main effect of RPL postpartum (POST), the main effect of wk, and their interactions. There was an effect of wk for all variables and therefore was not included in the table.⁵EBAL = energy balance; calculated based on NRC (2001).⁶Data were collected on 7 ± 0.71 and 14 ± 0.57 d relative to calving (DRC).⁷Data were collected on 7 ± 1.34 , 14 ± 1.41 , and 28 ± 1.13 DRC.⁸De novo fatty acids (FA) consist of C4 to C15, preformed FA consist of C18 and longer, and mixed FA consist of C16:0, C16:1, and C17:0 (Wojciechowski and Barbano, 2016).

Intake and BW Prepartum

Because of many factors, such as mammogenesis and conceptus growth, it is challenging to quantify the MP requirement and balance of IAA needed for the prepartum cow and how that may affect early lactation (Bell et al., 2000). The addition of RPL did not affect DMI of cows during the prepartum period, which is in agreement with studies supplementing RPL to lactating cows and dry cows (Misciattelli et al., 2003; Socha et al., 2005; Giallongo et al., 2016). Dissimilarly, when RPL was provided at 7.02% of MP and 1,296 g/d of MP, DMI increased in dry cows (Girma et al., 2019). Girma et al. (2019) postulated that increased

Lys metabolism increased carnitine synthesis, thereby increasing DMI and subsequently decreasing NEFA. Although DMI was not different with supply of RPL in the current study, cows in PRE-L had greater BW when compared with cows in PRE-C in the wk -2 and -1 before calving. However, there was no difference in the BW change from wk -4 to -1 and no difference in EBAL and BCS, suggesting that there may be limited biological significance to the difference in BW. In addition, plasma concentrations of 3-methylhistidine were not affected by treatment, suggesting that muscle mobilization was not required to maintain energy balance (Carder and Weiss, 2017). In previous studies in which RPL was supplemented at 1.27 g/kg of DMI and

Table 5. Least squares means and SEM for plasma AA (μM) of Holstein cows fed a common diet with rumen-protected Lys (RPL) or without (control) for 4 wk before calving

Variable ¹	Treatment ²		SEM ³	P-value ⁴		
	PRE-L	PRE-C		PRE	DRC	PRE $\times$ DRC
Total AA	1,810	1,920	33	0.02	<0.01	0.24
Total indispensable AA	719	752	13.2	0.08	<0.01	0.21
Total dispensable AA	1,100	1,170	24	0.05	0.32	0.36
Total branched-chain AA	361	386	7.0	0.02	<0.01	0.37
Total sulfur AA	74.6	81.3	1.64	0.005	0.51	0.89
Total urea cycle AA	157	158	3.29	0.79	<0.01	0.07
Indispensable						
Arg	61.0	60.6	1.65	0.87	<0.01	0.15
His	55.7	61.6	1.34	0.003	<0.01	0.33
Ile	86.9	92.5	1.88	0.04	<0.01	0.20
Leu	118	125	2.25	0.02	<0.01	0.68
Lys	69.8	62.5	1.83	0.009	<0.01	0.002
Lys, % of total AA	4.00	3.31	0.01	<0.01	<0.01	<0.01
Lys, % of indispensable AA	10.6	9.01	0.01	<0.01	<0.01	0.003
Met	29.9	31.9	0.83	0.10	0.05	0.68
Phe	45.6	46.6	0.76	0.32	<0.01	0.20
Thr	73.4	79.3	2.04	0.05	<0.01	0.40
Trp	22.4	23.5	0.87	0.37	<0.01	0.75
Val	157	168	3.27	0.02	<0.01	0.34
Dispensable						
Ala	193	192	4.45	0.83	<0.01	0.11
Asn	30.3	32.2	0.87	0.12	<0.01	0.12
Asp	4.62	5.45	0.18	0.002	<0.01	0.58
Cys	11.2	11.0	0.33	0.68	<0.01	0.10
Gln	310	344	10.75	0.03	0.49	0.35
Glu	41.6	44.4	1.00	0.05	<0.01	0.50
Gly	331	341	9.84	0.52	<0.01	0.25
Pro	54.9	57.8	1.26	0.11	<0.01	0.20
Ser	84.9	88.7	2.50	0.28	<0.01	0.46
Tyr	37.9	41.2	0.91	0.01	<0.01	0.12
Metabolites						
3-Methyl histidine	12.9	13.2	0.41	0.66	<0.01	0.96
Citrulline	64.1	65.7	1.38	0.43	<0.01	0.06
Ornithine	31.4	32.5	0.99	0.45	<0.01	0.28
Taurine	33.5	38.5	1.14	0.003	<0.01	0.71

¹Data were collected at -7 ± 0.5 and 0 ± 0.5 d relative to calving (DRC); total branched-chain AA = Ile, Leu, and Val; total sulfur AA = Met, taurine, and Cys; total urea cycle AA = Arg, citrulline, and ornithine; Lys as a % of total AA = $\text{Lys } (\mu M) / [\text{Total AA } (\mu M) - \text{Lys } (\mu M)]$; Lys as a % of total indispensable AA = $\text{Lys } (\mu M) / [\text{Total indispensable AA } (\mu M) - \text{Lys } (\mu M)]$.

²Dietary treatments consisted of a top dress either with RPL (PRE-L) or without (control; PRE-C) consumed for 4 wk before calving in a carrier of 300 g of dried molasses.

³Greatest value for SEM within treatment.

⁴Main effect of RPL prepartum (PRE), the main effect of DRC, and their interaction.

Table 6. Least squares means and SEM for plasma AA profile (μM) of Holstein cows fed a common diet with rumen-protected Lys (RPL) or without (control) for 4 wk before calving and 4 wk after calving

Variable ¹	Treatment ²				SEM ³	P-value ⁴						
	PRE-L POST-L	PRE-L POST-L	PRE-C POST-L	PRE-C POST-C		PRE	POST	DRC	PRE × DRC	POST × DRC	PRE × POST × DRC	
Total AA	2,300	2,300	2,330	2,350	67	0.58	0.92	0.12	0.77	0.36	0.87	0.91
Total indispensable AA	862	886	875	912	31.3	0.53	0.34	0.01	0.64	0.55	0.83	0.74
Total dispensable AA	1,440	1,420	1,440	1,440	49	0.87	0.80	0.53	0.91	0.30	0.87	0.94
Total branched-chain AA	442	471	454	480	20.9	0.61	0.19	0.001	0.74	0.38	0.94	0.85
Total sulfur AA	92.2	93.2	96.7	101	3.26	0.06	0.40	0.04	0.83	0.50	0.60	0.60
Total urea cycle AA	168	170	168	162	5.6	0.52	0.71	<0.01	0.31	0.33	0.53	0.82
Indispensable												
Arg	64.4	65.3	64.7	65.2	2.30	0.98	0.74	0.02	0.82	0.26	0.94	0.63
His	49.3	51.5	50.4	55.9	2.32	0.23	0.10	0.02	0.30	0.46	0.49	0.42
Ile	111	122	116	120	6.2	0.76	0.24	<0.01	0.73	0.46	0.57	0.81
Leu	135	138	136	145	6.4	0.53	0.37	0.03	0.44	0.49	0.66	0.80
Lys	83.4	79.0	81.2	81.1	3.75	0.99	0.54	0.41	0.61	0.48	0.57	0.56
Lys, % of total AA	3.72	3.52	3.66	3.55	0.12	0.89	0.20	0.99	0.70	0.68	0.73	0.44
Lys, % of indispensable AA	10.5	9.61	10.5	9.69	0.26	0.88	<0.01	0.05	0.84	0.41	0.85	0.48
Met	35.9	37.2	39.0	41.1	1.55	0.02	0.27	0.52	0.36	0.54	0.79	0.81
Phe	47.9	49.7	51.7	53.8	1.71	0.02	0.23	0.28	0.86	0.68	0.92	0.74
Thr	93.5	100	95.9	97.7	4.09	0.95	0.27	0.22	0.77	0.68	0.51	0.56
Trp	35.1	34.5	34.3	34.4	1.18	0.70	0.84	<0.01	0.77	0.71	0.74	0.92
Val	205	212	201	215	9.7	0.95	0.28	<0.01	0.52	0.81	0.70	0.81
Dispensable												
Ala	251	235	241	241	11.6	0.83	0.48	<0.01	0.39	0.86	0.51	0.96
Asn	46.2	46.2	46.8	46.1	2.06	0.91	0.87	0.02	0.89	0.38	0.88	0.94
Asp	6.33	6.47	6.90	6.79	0.37	0.23	0.97	<0.01	0.93	0.50	0.74	0.62
Cys	15.2	15.2	13.9	14.5	0.55	0.08	0.56	<0.01	0.65	0.94	0.62	0.87
Gln	267	260	264	270	11.3	0.77	0.97	0.01	0.60	0.32	0.56	0.89
Glu	43.0	39.7	42.3	43.3	1.64	0.38	0.48	0.75	0.19	0.60	0.19	0.08
Gly	578	588	580	575	30.4	0.86	0.93	0.69	0.66	0.23	0.81	0.97
Pro	86.2	85.6	84.8	85.9	3.46	0.88	0.94	<0.01	0.47	0.70	0.81	0.68
Ser	110	103	113	110	5.6	0.36	0.36	0.68	0.93	0.52	0.78	0.69
Tyr	40.5	42.1	42.8	43.0	1.58	0.31	0.57	0.01	0.97	0.60	0.68	0.91
Metabolites												
3-Methyl histidine	8.16	8.86	8.63	8.69	0.55	0.79	0.48	<0.01	0.32	0.70	0.56	0.99
Ornithine	28.8	27.6	28.7	28.7	1.28	0.69	0.63	<0.01	0.37	0.76	0.66	0.43
Taurine	41.4	40.9	40.4	45.4	2.28	0.43	0.32	0.17	0.26	0.52	0.24	0.54
Citrulline	74.5	77.2	74.5	68.3	3.25	0.17	0.58	<0.01	0.14	0.44	0.17	0.67

¹Data were collected at 7 ± 0.9 and 14 ± 0.9 d relative to calving (DRC); total branched-chain AA = Ile, Leu, and Val; total sulfur AA = Met, taurine, and Cys; total urea cycle AA = Arg, citrulline, and ornithine; Lys as a % of total AA = Lys (μM)/[Total AA (μM)]; Lys as a % of total indispensable AA = Lys (μM)/[Total indispensable AA (μM) - Lys (μM)].

²Dietary treatments included at top dress with RPL prepartum and postpartum (PRE-L POST-L), with RPL prepartum and without RPL postpartum (PRE-L POST-C), without RPL prepartum and with RPL postpartum (PRE-C POST-L), and without RPL prepartum and postpartum (control; PRE-C POST-C) in a carrier of 300 g of dried molasses.

³Greatest value for SEM within treatment.

⁴Consists of the main effect of RPL prepartum (PRE), the main effect of RPL postpartum (POST), the main effect of DRC, and their interactions.

27.6 g/cow of intestinally available Lys, respectively, DMI was not altered and there were no differences in BW (Robinson et al., 1999; Giallongo et al., 2016); however, both studies used lactating dairy cows and included a lower amount of RPL when compared with the current study. Additionally, the Lys:Met ratios were 3.02 (Robinson et al., 1999) and 2.99 (Giallongo et al., 2016). Compared with the 2 aforementioned studies, intestinally available Lys and Met were greater with a lower Lys:Met ratio in the present study. It is possible that Lys was not limiting for mid-lactation cows that were used by Robinson et al. (1999) and Giallongo et al. (2016) but was for prepartum cows in the present study. In the current study, plasma concentrations of Lys increased, whereas total AA decreased when RPL was consumed and DMI was similar, suggesting that increased Lys allowed for greater usage of other AA, though this is discussed more in depth in a later section. This may allow for balancing of AA during the prepartum period (Robinson, 2010). Alternatively, increased plasma concentrations of Lys may indicate no need for additional circulating Lys. Though this is possible, it is also probable that increased Lys was circulating to be utilized by other tissues. Previous research indicated that increased concentrations of limiting AA via rumen-protected AA supply corresponded with increased concentrations in follicular fluid (Acosta et al., 2017), liver (Zhou et al., 2017), and placental transporters (Batistel et al., 2017), indicating usage by those tissues.

Intake, BW, BCS, and BHB Postpartum

After calving, increased BW and a tendency for increased DMI were due to consumption of RPL prepartum. As evidence, the greater BW in cows consuming RPL prepartum allowed for cows to maintain BW postpartum and had a tendency for increased DMI postpartum. This is similar to Girma et al. (2019) and Xu et al. (1998) who also observed increased DMI postpartum when RPL was provided at 27 g/cow or 40 g/cow, respectively, of intestinally available Lys; however, these researchers also observed increased DMI prepartum. However, a dissimilar outcome was also reported, as RPL and RPM were consumed during the transition period, there were no changes in DMI and BW (Lee et al., 2019). It is possible that differences in diet formulation likely contributed to differences in results. For example, Lee et al. (2019) included starch at 28% of DM in the postpartum diet. This is greater than the current study in which starch was included at 24% of DM for the postpartum diet and 14% of DM for the prepartum diet. Though it is not provided in Lee et al. (2019), it is likely that the starch content was less

prepartum and resulted in a greater change from pre- to postpartum, possibly eliciting increased metabolic disorders (Drackley, 1999), which could contribute in explaining why DMI and BW were unchanged postpartum, dissimilar to the current study. Socha et al. (2005) supplemented pregnant dry cows for 2 wk before calving and 15 wk after calving and did not see any changes in DMI with RPL and RPM supplementation when provided at a Lys:Met ratio of 3.56. It was suggested by Zhou et al. (2016) that a Lys:Met ratio less than 3.0:1.0 may enhance DMI postpartum, which may explain the tendency for increased DMI in the current study due to adequate balancing of IAA prepartum. In a quantitative review conducted to determine the effect of RPL on production parameters, DMI was not different in lactating cows supplemented with RPL and RPM when compared with cows consuming a control diet but did not include data from transition cow studies where RPL was provided prepartum (Robinson, 2010). This is supported by other studies in which cows during early to mid-lactation did not receive prepartum RPL and changes in DMI were minimal (Misciattelli et al., 2003; Weiss, 2019). Additionally, RPL consumed by lactating cows only increased DMI when MP was deficient in the control diet (Giallongo et al., 2016). Variability in results reported with RPL supplementation could in part be due to RPL protection method, leading to differences in bioavailability of Lys. Although, it is likely that providing an optimal balance of Lys and thereby improving the overall balance of IAA prepartum resulted in increased voluntary intake postpartum. This result may be most apparent during the periparturient period due to the negative protein balance and physiological stress at this time (Osorio et al., 2013).

In addition to greater BW and a tendency for increased DMI postpartum, BHB was less at wk 1 postpartum in cows in PRE-L compared with cows in PRE-C but was not different by wk 2, suggesting that RPL provided prepartum is beneficial at the onset of lactation. In a transition cow study, postpartum BHB concentrations was not affected by prepartum consumption of RPL and RPM, but the percentage of MP provided by Lys and Met was less (6.03 and 1.89, respectively) than the current study (Lee et al., 2019). However, postpartum BHB decreased when RPL was consumed prepartum and postpartum or RPL and RPM were consumed postpartum (Girma et al., 2019; Lee et al., 2019). Beta-hydroxybutyrate is commonly used as a measure of oxidation of fat in detection of ketosis due to mobilization of body stores (Duffield, 2000). Blood concentrations of BHB in the current study were less than the threshold for subclinical ketosis and incidence of subclinical ketosis was not affected by consumption of RPL, which

may be due to a low number of cows that developed subclinical ketosis and therefore differences could not be detected between treatments.

Milk Yield and Composition Postpartum

A lack of a difference in milk yield when RPL is consumed by dairy cows is consistent with the literature (Misciattelli et al., 2003; Swanepoel et al., 2010; Girma et al., 2019). Balancing of AA is less likely to alter milk yield compared with milk protein (NRC, 2001; Robinson, 2010; Lee et al., 2019). Milk protein yield is enhanced with increased MP provided (Cant et al., 2018), which was detected in the current study. In addition to this, milk fat yield was also increased which would explain the increase in ECM yield due to prepartum consumption of RPL. This is not in agreement with the findings of Lee et al. (2019), in which cows consuming RPL and RPM during the prepartum and postpartum periods did not have milk yield responses. Differences between the findings of Lee et al. (2019) and the current study are most likely attributed to an increased supply of MP, in which their diet was formulated to provide 800 g/d of MP prepartum. In the current study, MP was provided at 1,190 g/d prepartum, likely providing adequate MP for mammogenesis along with maintenance requirements (Bell et al., 2000). By meeting or exceeding MP requirements prepartum, cows in the current study were able to adapt to early lactation more efficiently, as indicated by increased ECM yield and a tendency for increased DMI. Alternatively, cows consuming MP deficient diets supplemented with RPM, RPL, and rumen-protected histidine did not have a difference in milk yield compared with cows consuming a MP adequate diet, suggesting that supplementing a MP deficient diet with IAA can correct for the deficiency (Giallongo et al., 2016). However, in a study in which casein was infused during the transition period, protein absorption in the mammary gland of cows receiving the infusion increased without an increase in blood flow along the mammary gland (Larsen et al., 2014). This indicates that protein supply is the limiting factor for absorption of protein in the mammary gland (Larsen et al., 2014). Increased protein supply would increase protein yield and also increase milk yield and the requirement for lactose synthesis, which was detected in our study (Larsen et al., 2014). Other authors reported that rumen-protected histidine was a limiting AA for milk production in dairy cows, alongside Lys and Met, and by balancing for these 3 IAA cows were able to increase ECM in the current study (Schwab and Broderick, 2017).

When cows were supplemented with RPL during early to mid-lactation, there were no changes in milk

composition yields but there was an increase in milk true protein content (Misciattelli et al., 2003; Giallongo et al., 2016). Additionally, when cows consumed RPL prepartum and postpartum, there were no differences in milk composition content nor yields (Girma et al., 2019). Though 3-methylhistidine and EBAL were not different between treatments, a negative EBAL suggested that mobilization of body reserves did occur. When evaluating mid lactation dairy cows consuming RPM and rumen-protected histidine, Giallongo et al. (2015) reported differences in muscle AA. Furthermore, in the mammary gland, Lys is considered a Group II AA, meaning that it is taken up in excess of requirements for milk protein production and is catabolized to BCAA and DAA (Lapierre et al., 2009). One could hypothesize that in the current experiment when mobilization of body reserves occurred, the composition of AA mobilized from the muscle was different due to RPL; thereby, altering the AA entering circulation and providing the AA profile needed for increased milk composition yields. However, this hypothesis needs to be investigated further in regards to RPL.

Milk fat yield likely increased due to more available energy. Contrarily, when energy is limiting, milk fat yield is likely to decrease. Notably, cows in PRE-C POST-C are the most affected by this due to low intake resulting in less available energy, which resulted in the least yield of milk fat. This is further exemplified by de novo, mixed, and preformed FA detected in milk fat. During a negative EBAL, preformed FA in milk are more likely to be greater than de novo and mixed FA due to mobilization of body stores for energy. As DMI increases during early lactation, preformed FA decreases and de novo FA increases as a result of increased acetate production that occurs in the rumen and decreased plasma NEFA (Palmquist et al., 1993; Barbano et al., 2019). Though de novo FA were not different among treatments in the current study, mixed FA were greater for cows consuming RPL prepartum, and typically de novo FA are a greater contributor to mixed FA (Woolpert et al., 2016). Additionally, preformed FA were lower in cows that consumed RPL pre- and postpartum, indicating that consumption of RPL resulted in decreased mobilization of body stores to supply fat for milk fat. Body condition score, a reliable indicator of mobilization of body stores, was greater in cows consuming RPL postpartum, providing evidence that there was less mobilization (Roche et al., 2009). Enhanced efficiency is also indicated by a lesser conversion of feed to milk yield for cows in PRE-L POST-L. This further indicates that RPL consumption enhances efficiency during the transition period. It is also possible that increasing RPL in the diet allowed for adequate balancing and increased uptake of AA by the mammary

gland, noted specifically by decreased plasma concentrations of the IAA Val and His in the current study. Similar to Li et al. (2016), the difference in the IAA ratio may have increased mammary gland epithelium mRNA expression of specific lipogenic genes related to FA metabolism (i.e., acyl-CoA synthetase short-chain family member 2) and de novo FA synthesis (i.e., fatty acid synthase). However, further research, potentially involving mammary gland biopsies, is needed to verify this hypothesis as it relates to RPL. In this study, SCC was higher at wk 1 in cows consuming PRE-L POST-L compared with others, which was an unexpected result, though may have been strongly driven by a single cow with a large SCC at calving and therefore the physiological significance is likely limited.

Plasma AA and Metabolites

Increases in plasma Lys indicate an increase in the supply of Lys when RPL is consumed. This is demonstrated in the current study in which prepartum concentrations of Lys in plasma increased when RPL was consumed. In a similar study, there was a tendency for increased concentrations of Lys in plasma prepartum; however, intestinally supplied Lys was estimated at 61 g/d (Lee et al., 2019), whereas it was 98 g/d in the current study. Dissimilarly, during the postpartum period, plasma concentrations of Lys due to postpartum consumption of RPL were only increased when calculated as a percentage of IAA; but plasma concentrations are representative of absorption and catabolism and utilization of those AA (Meijer et al., 1994). Therefore, no change in plasma concentrations of Lys postpartum could indicate greater usage of this AA, specifically in the mammary gland (Larsen et al., 2015). This disagrees with other transition cow studies (Xu et al., 1998; Lee et al., 2019); however, plasma was collected on d 7 and 14 after calving in the current study and the aforementioned studies collected plasma for Lys concentration on d 56 and 22 after calving, respectively. Additionally, variability in protection methods for rumen-protected products would also result in differing bioavailability which may account for differences in results between studies. It is likely that a lack of increase of plasma concentration of Lys in cows consuming RPL postpartum occurred due to a decreased protein balance at this time compared with later in lactation. A negative protein balance is greatest from calving through 7 DIM and is positive by 28 DIM (Phillips et al., 2003). It has been suggested that supplying a greater amount of MP prepartum and during early lactation results in a more pronounced negative protein balance around 7 DIM, likely due to mammary and tissue need for protein (Larsen et al., 2014). Though there was also

decreased DMI accompanying the greater MP supply reported by Larsen et al. (2014), which would result in decreased energy intake and exacerbate the negative protein balance. Additionally, when AA were infused during the transition period, uptake of Lys was greatest during the first week of lactation, indicating that Lys is likely most limiting at this time (Larsen et al., 2015). However, plasma concentrations of 3-methylhistidine postpartum were not affected by treatment, suggesting that there is no difference in muscle catabolism (Carder and Weiss, 2017). If Lys is most limiting and it is being used for dispersion to tissues and increased uptake by the mammary gland, this would result in decreased plasma concentrations of AA. Under this assumption, it is probable that decreased plasma concentrations of total IAA and total DAA prepartum are likely due to increased ability to use these AA due to increased duodenal supply of Lys.

CONCLUSIONS

Because mammary gland growth begins during late gestation and continues into early lactation, it is possible that previous approximations of IAA required during the transition period have been underestimated. In the current study, prepartum consumption of RPL had the largest effects on postpartum performance and efficiency. This is exemplified by increased ECM and milk fat, protein, lactose, and casein yields, and a tendency for increased DMI during the postpartum period of cows that consumed RPL prepartum. Additionally, feeding RPL proved to be an adequate method to increase the concentration of Lys in plasma prepartum; however, this did not occur postpartum. This increase in concentration of Lys in plasma prepartum decreased many other IAA and DAA when RPL was consumed prepartum, suggesting that Lys was most limiting at this time. Therefore, the increasing concentrations of Lys in the plasma resulted in greater usage of IAA and DAA.

ACKNOWLEDGMENTS

This project was partially supported by Ajinomoto CO. Inc. (Tokyo, Japan) and by the USDA National Institute of Food and Agriculture (Washington, DC; NC-2042). We thank Adisseo (Alpharetta, GA) for the donation of Smartamine M; Dairy Nutrition Plus (Ralston, IA) for the donation of Soyplus; Phibro Animal Health Corporation (Teaneck, NJ) for the donation of Animate; and Perdue AgriBusiness (Salisbury, MD) for the donation of ProvAA1 AADvantage during the course of the experiment. Sincere appreciation is expressed to the Dairy Focus Team at the University

of Illinois (Urbana, IL), along with the University of Illinois Dairy Research Unit staff for assisting with data collection and cow health. Coauthors Sugimoto and Shinzato had input in the experimental design. The authors have not stated any conflicts of interest.

REFERENCES

- Acosta, D. A. V., M. I. Rivelli, C. Skenandore, Z. Zhou, D. H. Keisler, D. Luchini, M. N. Correa, and F. C. Cardoso. 2017. Effects of rumen-protected methionine and choline supplementation on steroidogenic potential of the first postpartum dominant follicle and expression of immune mediators in Holstein cows. *Theriogenology* 96:1–9. <https://doi.org/10.1016/j.theriogenology.2017.03.022>.
- AOAC International. 1995a. AOAC Official Method 934.01. Moisture in animal feed. 16th ed. Vol. 2. AOAC International, Arlington, VA.
- AOAC International. 1995b. AOAC Official Method 972.16. Fat, lactose, protein, and solids in milk. Mid-infrared spectroscopic method. 16th ed. Vol. 2. AOAC International, Arlington, VA.
- AOAC International. 1995c. AOAC Official Method 989.05. Ether extraction. 16th ed. Vol. 2. AOAC International, Arlington, VA.
- AOAC International. 1995d. AOAC Official Method 991.22. True protein. 16th ed. Vol. 2. AOAC International, Arlington, VA.
- AOAC International. 1995e. AOAC Official Method 990.20. Total solids. 16th ed. Vol. 2. AOAC International, Arlington, VA.
- Armenta, J. M., D. F. Cortes, J. M. Pisciotto, J. L. Shuman, K. Blakeslee, D. Rosoloso, O. Ogunbiyi, D. J. Sullivan, and V. Shulaev. 2010. Sensitive and rapid method for amino acid quantitation in malaria biological samples using AccQ•Tag ultra performance liquid chromatography-electrospray ionization-MS/MS with multiple reaction monitoring. *Anal. Chem.* 82:548–558. <https://doi.org/10.1021/ac901790q>.
- Barbano, D. M., C. Melilli, H. Dann, and R. Grant. 2019. The use of milk fatty acids as an indication of energy balance in dairy cows. Pages 52–70 in *Proc. Ruminant Nutr. Symp.* University of Florida, Gainesville, FL.
- Bartier, A. L., M. C. Windeyer, and L. Doepel. 2015. Evaluation of on-farm tools for colostrum quality measurement. *J. Dairy Sci.* 98:1878–1884. <https://doi.org/10.3168/jds.2014-8415>.
- Batistel, F., A. S. M. Alharthi, L. Wang, C. Parys, Y.-X. Pan, F. C. Cardoso, and J. J. Looor. 2017. Placental nutrient transporters and mammalian target of rapamycin signaling proteins are altered by the methionine supply during late gestation in dairy cows and are associated with newborn birth weight. *J. Nutr.* 147:1640–1647. <https://doi.org/10.3945/jn.117.251876>.
- Bell, A. W., W. S. Burhans, and T. R. Overton. 2000. Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. *Proc. Nutr. Soc.* 59:119–126. <https://doi.org/10.1017/S0029665100000148>.
- Cant, J. P., J. J. M. Kim, S. R. L. Cieslar, and J. Doelman. 2018. Symposium review: Amino acid uptake by the mammary glands: Where does the control lie? *J. Dairy Sci.* 101:5655–5666. <https://doi.org/10.3168/jds.2017-13844>.
- Carder, E. G., and W. P. Weiss. 2017. Short- and longer-term effects of feeding increased metabolizable protein with or without an altered amino acid profile to dairy cows immediately postpartum. *J. Dairy Sci.* 100:4528–4538. <https://doi.org/10.3168/jds.2016-12362>.
- Chalupa, W., and C. J. Sniffen. 1991. Protein and amino acid nutrition of lactating dairy cattle. *Vet. Clin. North Am. Food Anim. Pract.* 7:353–372. [https://doi.org/10.1016/S0749-0720\(15\)30798-2](https://doi.org/10.1016/S0749-0720(15)30798-2).
- Connell, A., A. G. Calder, S. E. Anderson, and G. E. Lobley. 1997. Hepatic protein synthesis in the sheep: Effect of intake as by use of stable-isotope-labelled glycine, leucine, and phenylalanine. *Br. J. Nutr.* 77:255–271. <https://doi.org/10.1079/BJN19970028>.
- Drackley, J. K. 1999. ADSA foundation scholar award: Biology of dairy cows during the transition period: The final frontier? *J. Dairy Sci.* 82:2259–2273. [https://doi.org/10.3168/jds.S0022-0302\(99\)75474-3](https://doi.org/10.3168/jds.S0022-0302(99)75474-3).
- Duffield, T. 2000. Subclinical ketosis in lactating dairy cattle. *Vet. Clin. North Am. Food Anim. Pract.* 16:231–253. [https://doi.org/10.1016/S0749-0720\(15\)30103-1](https://doi.org/10.1016/S0749-0720(15)30103-1).
- Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. *J. Dairy Sci.* 77:2695–2703. [https://doi.org/10.3168/jds.S0022-0302\(94\)77212-X](https://doi.org/10.3168/jds.S0022-0302(94)77212-X).
- Giallongo, F., M. T. Harper, J. Oh, J. C. Lopes, H. Lapierre, R. A. Patton, C. Parys, I. Shinzato, and A. N. Hristov. 2016. Effects of rumen-protected methionine, lysine, and histidine on lactation performance of dairy cows. *J. Dairy Sci.* 99:4437–4452. <https://doi.org/10.3168/jds.2015-10822>.
- Giallongo, F., A. N. Hristov, J. Oh, T. Frederick, H. Weeks, J. Werner, H. Lapierre, R. A. Patton, A. Gehman, and C. Parys. 2015. Effects of slow-release urea and rumen-protected methionine and histidine on performance of dairy cows. *J. Dairy Sci.* 98:3292–3308. <https://doi.org/10.3168/jds.2014-8791>.
- Girma, D. D., L. Ma, F. Wang, Q. R. Jiang, T. R. Callaway, J. K. Drackley, and D. P. Bu. 2019. Effects of close-up dietary energy level and supplementing rumen-protected lysine on energy metabolites and milk production in transition cows. *J. Dairy Sci.* 102:7059–7072. <https://doi.org/10.3168/jds.2018-15962>.
- Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism feeding the transition dairy cow. *J. Anim. Sci.* 73:2820–2833. <https://doi.org/10.2527/1995.7392820x>.
- Kononoff, P. J., A. J. Heinrichs, and D. R. Buckmaster. 2003. Modification of the Penn State forage and total mixed ration particle separator and the effects of moisture content on its measurements. *J. Dairy Sci.* 86:1858–1863. [https://doi.org/10.3168/jds.S0022-0302\(03\)73773-4](https://doi.org/10.3168/jds.S0022-0302(03)73773-4).
- Krause, K. M., D. V. Dhuyvetter, and G. R. Oetzel. 2009. Effect of a low-moisture buffer block on ruminal pH in lactating dairy cattle induced with subacute ruminal acidosis. *J. Dairy Sci.* 92:352–364. <https://doi.org/10.3168/jds.2007-0959>.
- Lapierre, H., R. Berthiaume, G. Raggio, M. C. Thivierge, L. Doepel, D. Pacheco, P. Dubreuil, and G. E. Lobley. 2005. The route of absorbed nitrogen into milk protein. *Anim. Sci.* 80:11–22. <https://doi.org/10.1079/ASC41330011>.
- Lapierre, H., L. Doepel, E. Milne, and G. E. Lobley. 2009. Responses in mammary and splanchnic metabolism to altered lysine supply in dairy cows. *Animal* 3:360–371. <https://doi.org/10.1017/S1751731108003571>.
- Larsen, M., C. Galindo, D. R. Ouellet, G. Maxin, N. B. Kristensen, and H. Lapierre. 2015. Abomasal amino acid infusion in postpartum dairy cows: Effect on whole-body, splanchnic, and mammary amino acid metabolism. *J. Dairy Sci.* 98:7944–7961. <https://doi.org/10.3168/jds.2015-9439>.
- Larsen, M., H. Lapierre, and N. B. Kristensen. 2014. Abomasal protein infusion in postpartum transition dairy cows: Effect on performance and mammary metabolism. *J. Dairy Sci.* 97:5608–5622. <https://doi.org/10.3168/jds.2013-7247>.
- Lee, C., N. E. Lobos, and W. P. Weiss. 2019. Effects of supplementing rumen-protected lysine and methionine during prepartum and postpartum periods on performance of dairy cows. *J. Dairy Sci.* 102:11026–11039. <https://doi.org/10.3168/jds.2019-17125>.
- Li, P., Y. L. Yin, D. Li, S. Woo Kim, and G. Wu. 2007. Amino acids and immune function. *Br. J. Nutr.* 98:237–252. <https://doi.org/10.1017/S000711450769936X>.
- Li, S., A. Hosseini, M. Danes, C. Jacometo, J. Liu, and J. J. Looor. 2016. Essential amino acid ratios and mTOR affect lipogenic gene networks and miRNA expression in bovine mammary epithelial cells. *J. Anim. Sci. Biotechnol.* 7:44. <https://doi.org/10.1186/s40104-016-0104-x>.
- Littell, R. C. 2002. Analysis of unbalanced mixed model data: A case study comparison of ANOVA versus REML/GLS. *J. Agric. Biol. Environ. Stat.* 7:472–490. <https://doi.org/10.1198/108571102816>.
- McDowell, D., N. Hooven, and K. Camoens. 1976. Effects of climate on performance of Holsteins in first lactation. *J. Dairy Sci.* 59:965–971. [https://doi.org/10.3168/jds.S0022-0302\(76\)84305-6](https://doi.org/10.3168/jds.S0022-0302(76)84305-6).
- Meijer, G. A. L., J. Van Der Meulen, J. G. M. Bakker, C. J. Van Der Koelen, and A. M. Van Vuuren. 1995. Free amino acids in

- plasma and muscle of high yielding dairy cows in early lactation. *J. Dairy Sci.* 78:1131–1141. [https://doi.org/10.3168/jds.S0022-0302\(95\)76730-3](https://doi.org/10.3168/jds.S0022-0302(95)76730-3).
- Misciattelli, L., V. F. Kristensen, M. Vestergaard, M. R. Weisbjerg, K. Sejrsen, and T. Hvelplund. 2003. Milk production, nutrient utilization, and endocrine responses to increased postruminal lysine and methionine supply in dairy cows. *J. Dairy Sci.* 86:275–286. [https://doi.org/10.3168/jds.S0022-0302\(03\)73606-6](https://doi.org/10.3168/jds.S0022-0302(03)73606-6).
- NRC (National Research Council). 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. The National Academies Press, Washington, DC.
- Osorio, J. S., P. Ji, J. K. Drackley, D. Luchini, and J. J. Loor. 2013. Supplemental Smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. *J. Dairy Sci.* 96:6248–6263. <https://doi.org/10.3168/jds.2012-5790>.
- Overton, T. R., D. W. Lacount, T. M. Cicela, and J. H. Clark. 1996. Evaluation of a ruminally protected methionine product for lactating dairy cows. *J. Dairy Sci.* 79:631–638. [https://doi.org/10.3168/jds.S0022-0302\(96\)76408-1](https://doi.org/10.3168/jds.S0022-0302(96)76408-1).
- Palmquist, D. L., A. D. Bealieu, and D. M. Barbano. 1993. Feed and animal factors influencing milk fat composition. *J. Dairy Sci.* 76:1753–1771. [https://doi.org/10.3168/jds.S0022-0302\(93\)77508-6](https://doi.org/10.3168/jds.S0022-0302(93)77508-6).
- Phillips, G. J., T. L. Citron, J. S. Sage, K. A. Cummins, M. J. Cecava, and J. P. McNamara. 2003. Adaptations in body muscle and fat in transition dairy cattle fed differing amounts of protein and methionine hydroxy analog. *J. Dairy Sci.* 86:3634–3647. [https://doi.org/10.3168/jds.S0022-0302\(03\)73969-1](https://doi.org/10.3168/jds.S0022-0302(03)73969-1).
- Robinson, P. H. 2010. Impacts of manipulating ration metabolizable lysine and methionine levels on the performance of lactating dairy cows: A systematic review of the literature. *Livest. Sci.* 127:115–126. <https://doi.org/10.1016/j.livsci.2009.10.003>.
- Robinson, P. H., W. Chalupa, C. J. Sniffen, W. E. Julien, H. Sato, T. Fujieda, K. Watanabe, and H. Suzuki. 1999. Influence of postruminal supplementation of methionine and lysine, isoleucine, or all three amino acids on intake and chewing behavior, ruminal fermentation, and milk and milk component production. *J. Anim. Sci.* 77:2781–2792. <https://doi.org/10.2527/1999.77102781x>.
- Roche, J. R., N. C. Friggens, J. K. Kay, M. W. Fisher, K. J. Stafford, and D. P. Berry. 2009. Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. *J. Dairy Sci.* 92:5769–5801. <https://doi.org/10.3168/jds.2009-2431>.
- Schwab, C. G., and G. A. Broderick. 2017. A 100-year review: Protein and amino acid nutrition in dairy cows. *J. Dairy Sci.* 100:10094–10112. <https://doi.org/10.3168/jds.2017-13320>.
- Socha, M. T., D. E. Putnam, B. D. Garthwaite, N. L. Whitehouse, N. A. Kierstead, C. G. Schwab, G. A. Ducharme, and J. C. Robert. 2005. Improving intestinal amino acid supply of pre- and postpartum dairy cows with rumen-protected methionine and lysine. *J. Dairy Sci.* 88:1113–1126. [https://doi.org/10.3168/jds.S0022-0302\(05\)72778-8](https://doi.org/10.3168/jds.S0022-0302(05)72778-8).
- Sprecher, D. J., D. E. Hostetler, and J. B. Kaneene. 1997. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. *Theriogenology* 47:1179–1187. [https://doi.org/10.1016/S0093-691X\(97\)00098-8](https://doi.org/10.1016/S0093-691X(97)00098-8).
- Swanepoel, N., P. H. Robinson, and L. J. Erasmus. 2010. Amino acid needs of lactating dairy cows: Impact of feeding lysine in a ruminally protected form on productivity of lactating dairy cows. *Anim. Feed Sci. Technol.* 157:79–94. <https://doi.org/10.1016/j.anifeedsci.2010.02.008>.
- Vyas, D., and R. A. Erdman. 2009. Meta-analysis of milk protein yield responses to lysine and methionine supplementation. *J. Dairy Sci.* 92:5011–5018. <https://doi.org/10.3168/jds.2008-1769>.
- Weiss, W. P. 2019. Effects of feeding diets composed of corn silage and a corn milling product with and without supplemental lysine and methionine to dairy cows. *J. Dairy Sci.* 102:2075–2084. <https://doi.org/10.3168/jds.2018-15535>.
- Wojciechowski, K. L., and D. M. Barbano. 2016. Prediction of fatty acid chain length and unsaturation of milk fat by mid-infrared milk analysis. *J. Dairy Sci.* 99:8561–8570. <https://doi.org/10.3168/jds.2016-11248>.
- Woolpert, M. E., H. M. Dann, K. W. Cotanch, C. Melilli, L. E. Chase, R. J. Grant, and D. M. Barbano. 2016. Management, nutrition, and lactation performance are related to bulk tank milk de novo fatty acid concentration on northeastern US dairy farms. *J. Dairy Sci.* 99:8486–8497. <https://doi.org/10.3168/jds.2016-10998>.
- Wu, Z., R. J. Fisher, C. E. Polan, and C. G. Schwab. 1997. Lactational performance of cows fed low or high ruminally undegradable protein prepartum and supplemental methionine and lysine postpartum. *J. Dairy Sci.* 80:722–729. [https://doi.org/10.3168/jds.S0022-0302\(97\)75991-5](https://doi.org/10.3168/jds.S0022-0302(97)75991-5).
- Xu, S., J. H. Harrison, W. Chalupa, C. Sniffen, W. Julien, H. Sato, T. Fujieda, K. Watanabe, T. Ueda, and H. Suzuki. 1998. The effect of ruminal bypass lysine and methionine on milk yield and composition of lactating cows. *J. Dairy Sci.* 81:1062–1077. [https://doi.org/10.3168/jds.S0022-0302\(98\)75668-1](https://doi.org/10.3168/jds.S0022-0302(98)75668-1).
- Zhou, Z., M. Vailati-Riboni, E. Trevisi, J. K. Drackley, D. N. Luchini, and J. J. Loor. 2016. Better postpartal performance in dairy cows supplemented with rumen-protected methionine compared with choline during the periparturition period. *J. Dairy Sci.* 99:8716–8732. <https://doi.org/10.3168/jds.2015-10525>.
- Zhou, Z., E. Trevisi, D. N. Luchini, and J. J. Loor. 2017. Differences in liver functionality indexes in periparturition dairy cows fed rumen-protected methionine or choline are associated with performance, oxidative stress status, and plasma amino acid profiles. *J. Dairy Sci.* 100:6720–6732. <https://doi.org/10.3168/jds.2016-12299>.

ORCIDS

L. K. Fehlberg <https://orcid.org/0000-0002-2874-5966>

F. C. Cardoso <https://orcid.org/0000-0003-1025-3231>