Effects of increased doses of lysine in a rumen-protected form on plasma amino acid concentration and lactational performance of dairy cows fed a lysine-deficient diet

V. M. R. Malacco,^{1,2} L. Beckett,¹ S. Hilger,¹ P. Doane,³ R. B. Reis,² and S. S. Donkin^{1*†}

¹Department of Animal Sciences, Purdue University, West Lafayette, IN 47907

²Department of Animal Science, Federal University of Minas Gerais, Minas Gerais, 30161-970, Brazil

³Archer Daniels Midland Company, ADM Research, Decatur, IL 62521

ABSTRACT

The objective of these studies was to determine the effects of feeding a novel rumen-protected Lys (RP-Lys) product on plasma AA, lactational performance, and Lys bioavailability. To evaluate RP-Lys on lactation performance a corn-based diet (42.5% of corn silage and 21.9% of corn and corn by-products, on DM basis) was formulated to be Lys deficient but adequate in Met, energy, and metabolizable protein. Thirty-six lactating Holstein cows were fed either a Lys-deficient control diet (CON) with no added RP-Lys, or diets containing 0.3% of RP-Lys (0.3RP-Lys) or 0.6% of RP-Lys (0.6RP-Lys) for 8 wk. There were no effects on dry matter intake (mean $\pm$ SD; 26.1 ± 0.58 kg/d), milk yield (37.9 ± 0.72 kg/d), or milk composition to the RP-Lys supplementation. No effect was observed on plasma AA concentrations except for His. Plasma His was linearly reduced by Lys feeding (42.6, 41.2, 30.0 ± 4.09 μ M, for CON, 0.3RP-Lys, and 0.6RP-Lys, respectively). Calculated efficiency of Lys utilization decreased linearly with RP-Lys supplementation. In the companion study, 3 rumen-cannulated lactating dairy cows were used in a 3×3 Latin square design to assess the bioavailability of the RP-Lys. Free Lys (HCl-Lys), RP-Lys, and water were administered separately by postprandial bolus dosing. The Lys bioavailability was assessed by the ratio of area under the curve of Lys plasma concentration for RP-Lys compared with HCl-Lys and discounted for the area under the curve for water bolus dose. The estimated bioavailability of the RP-Lys was $24.4\% \pm 4.61$. In summary, increased supplemental doses of Lys had no effect on Lys plasma concentration and lactational performance when fed to

dairy cows on a corn-based diet, although altered Lys as % of essential AA was observed. However, the lack of effects should be considered in light of the lower-than-expected bioavailability of the RP-Lys.

Key words: amino acid, protein, dairy cow, bioavailability

INTRODUCTION

The ideal protein provides the perfect balance of biologically available AA relative to the animal's physiological needs. Meeting the proteins needs of high-producing dairy cows is complex and involves accurately predicting different fractional contributions from rumen microbial protein and rumen undegraded protein to the absorbable AA pool, intestinal absorptive efficiency, and the peripheral tissue requirements for AA. Greater precision in meeting the AA needs of lactating dairy cows may increase productivity, reduce N excretion to the environment, or result in a combination of both factors (Castillo et al., 2000; Godden et al., 2001). Improved knowledge of protein and AA profiles of conventional feed ingredients combined with an understanding of the value of AA supplements in current feeding systems is necessary to advance feeding management strategies toward greater precision in meeting AA requirements for lactating cows.

In many parts of the United States, rations for dairy cows contain corn and corn-based products. Corn milling by-products are an economical source of NE_L and MP, but can lead to limitations in Lys supply due to the predominance of zein protein, a poor source of Lys (Coleman and Larkins, 1999; Mullins et al., 2013; Weiss, 2019). Thus, when corn and corn products comprise a significant proportion of the diets, dairy nutritionists have formulated rations using rumen-protected sources of Lys to meet Lys requirements for high-producing cows. Despite recognizing Lys is likely limiting, there is still a scarcity of research demonstrating consistent responses to rumen-protected (RP) Lys supplementa-

Received June 4, 2021.

Accepted November 29, 2021.

*Corresponding author: shawn.donkin@oregonstate.edu

†Current address: College of Agricultural Sciences, Oregon State University, Corvallis 97331.

tion on milk and milk components (Robinson et al., 2010). Although variable, positive responses in milk production and milk protein composition and yield were observed with RP Lys supplementation in early lactating cows (Socha et al., 2005) and mid-lactating cows (Bernard et al., 2014; Giallongo et al., 2016; Lobos et al., 2021). Small or nonsignificant responses to Lys supplementation were also reported in the literature (Arriola Apelo et al., 2014; Fehlberg et al., 2020; Morris and Kononoff, 2020).

The AA absorption process is multifaceted, involving numerous AA transporters; in ruminants, however, intestinal absorption of AA has not been reported to limit protein absorption (Bröer, 2008; Harmon and Swanson, 2020). Therefore, the success of Lys supplementation in RP form is dependent on the adequacy of protection against Lys breakdown by ruminal microorganisms and availability for absorption into blood by the intestine (Berthiaume et al., 2000). Amino acid bioavailability is defined as the percentage of intestinally available AA absorbed into the blood and available for utilization by tissues, which could be defined by the supply of AA from the digestive tract to the body tissue (Agricultural Research Council, 1981; Danfær and Fernández, 1999). One of the major implications that hinder dietary AA bioavailability is potential degradation in the rumen. Methods commonly used in the feed industry to protect individual or more purified AA sources from ruminal degradation include (1) feeding AA analogs, which are stable under ruminal conditions and are available to the cow; (2) coating AA with hydrogenated fat, proteins, mixtures of fats and proteins, or calcium soaps of long-chain fatty acids; and (3) encapsulation with polymer compounds resistant to ruminal degradation but are dissolved at the pH of the abomasum (Alves, 2004). Regardless of the method of protection, all RP AA should have the following attributes: rumen stability, intestinal digestibility, and high bioavailability.

Hydrogenated fatty acids are effective as a coating strategy to deliver AA postruminally because highly SFA are not subject to biohydrogenation in the rumen, remain insoluble at a normal range of rumen pH (5.5 to 7), and can be digested in the intestine, thus releasing AA (Behan et al., 2019). However, newly developed products purported to provide RP Lys must be evaluated for postruminal delivery of Lys that can be absorbed and utilized by the lactating dairy cow.

Studies on the effects of Lys supplementation in a RP form on milk and milk protein production in lactating dairy cows are available in the literature (Socha et al., 2005; Giallongo et al., 2016; Morris and Kononoff, 2020). However, to our knowledge, studies that assessed the *in vivo* bioavailability of the RP-Lys source coupled with a production response evaluation in lactating

cows are lacking. Thus, 2 companion experiments were designed to evaluate (1) the supplementation of incremental amounts of an RP-Lys product on plasma AA concentration, feed intake, and lactation performance of dairy cows fed a corn-based Lys-deficient diet and (2) determine the relative postruminal bioavailability of the RP-Lys product using an area under the curve approach following a postruminal bolus dose. We hypothesized that incremental Lys supplied through the feeding of RP-Lys source to cows fed with Lys-deficient diet would change plasma AA concentration, increase milk yield, and increase milk protein yield.

MATERIALS AND METHODS

The experiments were conducted between October and December 2018 at the Purdue University Dairy Research and Education Center, following animal care and handling protocols approved by the Purdue University Animal Care and Use Committee.

The RP-Lys product used in the following experiments is composed of 50% Lys hydrochloride, 49% hydrogenated soybean oil (87.8% C18:0 and 10.6% C16:0), and 1% of an emulsifier. The process used to protect the product involves mixing the AA with the coating mixture (hydrogenated fat and emulsifier) to form a slurry that is used to create the prills. The prills have a size range of 2.4 to 3.4 mm and an average of 3.21 mm (± 0.11 SD) of diameter. The RP-Lys has 39.3 g (± 0.9 SD) of Lys per 100 g of the product.

Experiment 1

The first experiment assessed the response of milk production and components to feeding RP-Lys in a Lys-deficient diet. Thirty-six Holstein dairy cows (6 primiparous and 30 multiparous), averaging (mean $\pm$ SD) 123 $\pm$ 32 DIM, 653 $\pm$ 67 kg BW, and 38.3 $\pm$ 5.0 kg of milk/d were blocked following a 7-d covariate period by milk yield and parity, and were randomly assigned to 1 of the 3 treatment groups ($n = 12$ cows per group) using a random number generator in Excel (Microsoft Corp.). Power analysis was conducted using proc power procedure of SAS (ver. 9.4, SAS Institute Inc.) to determine the sample size required to identify 2 standard deviations (12 μ M) of difference in plasma Lys concentration and the variation associated with the plasma Lys concentrations if present, at $P < 0.05$, power of 0.80. The plasma Lys concentration presented by Giallongo et al. (2016) indicated that a minimum sample size of 9 would be sufficient to detect that differences in plasma Lys concentrations with an actual power of 0.81. Cows were included in the study if they were <180 DIM and yielded more than 30 kg of milk

at the beginning of the study. Cows were moved to tie stalls and allowed to acclimate over a period of 7 d to the stalls. Dry matter intake, milk production, and milk composition were assessed during this period, and the weekly average was used as a covariate term.

During the experiment, cows were fed the treatment diets presented as a TMR once daily in amounts that ensured ad libitum consumption with a target of 10% feed refusals. Fresh water was freely provided. Cows were weighed weekly, and BCS was assigned according to a 5-point scale (Wildman et al., 1982) by 2 trained individuals after the morning milking. Cows were fed one of 3 treatment diets (Table 1) delivered as a TMR for 56 d.

The experimental diets were a control (**CON**) diet formulated to be deficient in digestible Lys (**dLys**) (i.e., dLys <6.6% of MP) and meeting NE_L and MP to support 40 kg of milk (NRC, 2001) and 2 diets with incremental levels of RP-Lys (Table 1 and 2). The RP-Lys supplemented diets consisted of the inclusion of RP-Lys ($39.3 \pm 0.9\%$ SD of Lys; NutraPass-50L, Archer Daniels Midland Co.) as 0.3% of diet DM (**0.3RP-Lys**) and as 0.6% of diet DM (**0.6RP-Lys**). The RP-Lys was incorporated in a grain mix that was added and thoroughly mixed into the TMR daily before feeding to the cows. The Met concentration of the basal diet was adjusted to achieve 2.2% of MP through addition of RP Met (Smartamine M, Adisseo Inc.). The cow's requirements for dMet, dLys, and dHis (Table 3) were calculated as 2.2, 6.6, and 2.2% of the MP requirements, respectively (NRC, 2001; Schwab et al., 2005; Lee et al., 2012). The experimental diets were color coded, and the codes and group allocation were under the responsibility of the study coordinator. The codes were not revealed to other animal caretakers.

Cows were released from tie stalls twice daily and milked at 0500 and 1600 h. Milk yield was measured at each milking using an automatic system (Afimilk animal monitoring system, version 5.3). Milk samples were collected during 2 consecutive milkings, in the evening and subsequent morning milking each week and analyzed for milk fat, protein, lactose, SCC (B2000 Infrared Analyzer, Bentley Instruments) and MUN (Skalar) by Dairy One (Ithaca, NY).

Dry matter intake was determined daily for each cow by subtracting refused feed from feed offered in the corresponding 24-h period. Samples of TMR and individual feed ingredients were collected weekly, dried in a forced-air oven at 60°C, and ground to pass through a 2-mm screen. Weekly feed samples were analyzed for DM content and used to adjust the proportions of diet ingredients on an as-fed basis. Weekly TMR and feed samples were analyzed by wet chemistry methods for CP (method 990.03; AOAC International, 2000),

crude fat (method 2003.05; AOAC International, 2006) amylase-treated NDF (Van Soest et al., 1991), ADF (method 973.18; AOAC International, 2000), ash (method 942.05; AOAC International, 2000), minerals (method 985.01; AOAC International, 2000), and NFC and NE_L was estimated by Dairy One (Ithaca, NY). The AA profile of the CON TMR diet and RP-Lys product were analyzed at Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO) following AOAC International methods (method

Table 1. Ingredient composition of experimental diets that either had no supplementation of a rumen-protected Lys (RP-Lys) product, or had 0.3% of diet DM or 0.6% of diet DM supplemented with a RP-Lys product fed to dairy cows (experiment 1)

Item, % of diet DM	Treatment ¹		
	CON	0.3RP-Lys	0.6RP-Lys
Corn silage ²	42.5	42.5	42.4
Alfalfa haylage ³	12.7	12.7	12.7
Rye hay ⁴	1.9	1.9	1.9
High-moisture corn	12.7	12.7	12.7
Dried distillers corn grain	7.6	7.6	7.6
Soybean hulls	7.2	7.2	7.2
Corn grain, ground	6.4	6.4	6.3
Corn gluten meal, dried	2.8	2.8	2.8
Soybean meal	2.3	2.3	2.2
Calcium carbonate	0.85	0.85	0.84
Sodium bicarbonate	0.85	0.85	0.84
Urea	0.51	0.51	0.51
Rumen-protected fat ⁵	0.5	0.5	0.51
Salt	0.3	0.3	0.29
Hydrogenated soybean oil ⁶	0.3	0.15	0
Calcium phosphate	0.21	0.2	0.21
Magnesium oxide	0.17	0.17	0.17
Rumen-protected Met ⁷	0.13	0.13	0.13
Vitamin and trace mineral mix ⁸	0.09	0.08	0.08
Mycotoxin binder ⁹	0.04	0.04	0.04
Monensin ¹⁰	0.01	0.01	0.01
Rumen-protected Lys ¹¹	0	0.3	0.6

¹CON = control diet with no RP-Lys; 0.3RP-Lys = diet supplemented with 0.3% of diet DM of RP-Lys; 0.6RP-Lys = diet supplemented with 0.6% of diet DM of RP-Lys.

²Corn silage was 31.8% DM and contained (DM basis) 8.2% CP and 35.7% NDF.

³Haylage was 32.9% DM and contained (DM basis) 19.3% CP and 39.1% NDF.

⁴Rye hay was 79.3% DM and contained (DM basis) 9.7% CP and 65.5% NDF.

⁵Enertia rumen bypass fat from palm oil (ADM Animal Nutrition).

⁶Hydrogenated soybean oil (87.8% C18:0 and 10.6% C16:0; Dritex S; ADM Animal Nutrition).

⁷Smartamine M (Adisseo Inc.).

⁸Purdue Dairy TMV premix: calcium, minimum 5.00%; calcium, maximum 7.00%; copper, minimum 12,000 mg/kg; selenium, minimum 260 mg/kg; zinc, minimum 54,500 mg/kg; manganese, minimum 44,500 mg/kg; iodine, minimum 440 mg/kg; cobalt, minimum 1,580 mg/kg; vitamin A, minimum 6,615,000 IU/kg; vitamin D, minimum 1,653,750 IU/kg; and vitamin E, minimum 30,870 IU/kg.

⁹ETX-5 (Feedworks USA Ltd.).

¹⁰Rumensin (Elanco).

¹¹RP-Lys (ADM Animal Nutrition).

994.12; AOAC International, 2000). Samples were analyzed once, and not in replicates.

Measurement of Blood AA and Metabolites

Blood samples were collected once on d 35 by coccygeal venipuncture into vacutainers containing sodium heparin (BD Diagnostics) 5 h after the morning feeding. Samples were inverted several times to ensure mixing of the anticoagulating agent and then kept on ice until centrifugation at $1,000 \times g$ for 15 min at 4°C. Centrifugation and plasma separation occurred within 30 min of sample collection. Harvested plasma was stored at -80°C until analysis. Plasma samples were analyzed for

Table 2. Nutrient composition of experimental diets that either had no supplementation of a rumen-protected Lys (RP-Lys) product, or had 0.3% of diet DM or 0.6% of diet DM supplemented with an RP-Lys product fed to dairy cows (experiment 1)

Item, ¹ % of DM	Treatment ²		
	CON	0.3RP-Lys	0.6RP-Lys
DM	45.3 ± 1.2	45.4 ± 1.6	45.3 ± 1.5
CP	16.0 ± 0.7	15.7 ± 0.8	16.2 ± 0.7
NDF	29.2 ± 2.4	30.0 ± 2.5	28.9 ± 1.7
Crude fat	4.3 ± 0.2	4.2 ± 0.3	4.1 ± 0.3
NFC	44 ± 2.4	43.8 ± 2.8	44.3 ± 2.7
Ash	6.5 ± 0.2	6.3 ± 0.5	6.5 ± 0.4
NE _L , ³ Mcal/kg	1.74 ± 0.04	1.73 ± 0.03	1.75 ± 0.01
Ca	0.62 ± 0.05	0.56 ± 0.07	0.63 ± 0.07
P	0.38 ± 0.02	0.38 ± 0.02	0.38 ± 0.02
Mg	0.28 ± 0.01	0.27 ± 0.02	0.26 ± 0.01
K	1.08 ± 0.06	1.06 ± 0.04	1.05 ± 0.02
Na	0.42 ± 0.03	0.39 ± 0.05	0.39 ± 0.03
AA, ⁴ g/kg of DM			
Ala	10.1	10.1	10.1
Arg	4.6	4.7	4.7
Asp	10.4	10.4	10.4
Cys	2.2	2.2	2.2
Glu	19.1	19.1	19.1
Gly	5.9	5.9	5.9
His	3.0	3.0	3.0
Ile	5.8	5.8	5.8
Leu	14.2	14.2	14.2
Lys	5.7	6.8	8.0
Met	3.5	3.5	3.5
Phe	6.9	6.9	6.9
Pro	9.5	9.5	9.5
Ser	5.3	5.3	5.3
Thr	5.0	5.0	5.0
Trp	1.0	1.0	1.0
Tyr	3.4	3.4	3.4
Val	7.2	7.2	7.2

¹Values are presented as an average of 5 samples ± SD.

²CON = control diet with no RP-Lys; 0.3RP-Lys = diet supplemented with 0.3% of diet DM of RP-Lys; 0.6RP-Lys = diet supplemented with 0.6% of diet DM of RP-Lys.

³Calculated (NRC, 2001) using treatment average DMI, BW, milk production and composition, and assayed feed composition.

⁴The CON diet was analyzed for AA composition, and the AA composition of the treatment diets was mathematically estimated.

Table 3. Protein fractions and AA balance in dairy cows fed with Lys-deficient diet that either had no supplementation of a rumen-protected Lys (RP-Lys) product, or had 0.3% of diet DM or 0.6% of diet DM supplemented with a RP-Lys product (experiment 1)

Item ¹	Treatment ²		
	CON	0.3RP-Lys	0.6RP-Lys
Protein fraction balance, g/d			
MP			
Requirements	2,658	2,613	2,615
Supply	2,828	2,829	2,845
Balance	170	216	230
MP allowable milk, kg/d	42.9	42.4	41.8
RDP and RUP			
RDP supply	2,454	2,431	2,441
RDP balance	-128	-126	-123
RUP supply	1,723	1,740	1,754
RUP balance	214	272	289
AA balance, g/d			
dLys			
Requirements ⁴	175	172	173
Supply from the diet	156	155	155
Supply from RP-Lys ⁵	0	20	39
Balance	-19	3	21
dMet			
Requirements ⁴	58	57	58
Supply	75	71	72
Balance	17	14	14
dHis			
Requirements ⁴	58	57	58
Supply	59	58	58
Balance	1	1	0

¹All values were estimated using NRC (2001) based on actual averaged DMI, milk yield and composition, and BW of individual cows during 8 wk of data collection. Due to rounding, balance may not exactly match requirements and supply.

²CON = control diet with no RP-Lys; 0.3RP-Lys = diet supplemented with 0.3% of diet DM of RP-Lys; 0.6RP-Lys = diet supplemented with 0.6% of diet DM of RP-Lys.

⁴Requirements of dMet, dLys, and dHis were calculated as 2.2, 6.6, and 2.2%, respectively, of MP requirements (NRC, 2001; Schwab et al., 2005; Lee et al., 2012). dMet, dLys, and dHis = digestible Met, Lys, and His, respectively.

⁵Supply of dLys from RP-Lys was estimated from Lys content and bioavailability data: 39.3% Lys and 63.1% bioavailability.

AA concentration at the University of Missouri–Columbia Agricultural Experiment Station Chemical Laboratory (Columbia, MO; Deyl et al., 1986; Fekkes, 1996). Samples were analyzed once, and not in replicates.

Calculations

Weekly BW and BCS changes were calculated for each cow using the BW and BCS of the previous week. Milk protein, fat, solids, and lactose yield were calculated from milk yield and milk composition data collected weekly. Energy corrected milk (kg/d) was calculated as ECM = kg of milk × [(38.3 × % fat × 10 + 24.2 × % true protein × 10 + 16.54 × % lactose × 10 + 20.7) ÷ 3,140] (Sjaunja et al., 1991). The efficiency of N utilization for milk protein production was calculated

as N yield in milk true protein divided by N intake $\times$ 100 and milk N yield was calculated from the milk true protein yield (kg) divided by a factor of 6.38. Daily DMI, milk production and milk composition data were averaged weekly for each cow. The efficiency of Lys use was calculated as described in Omphalius et al. (2020). Briefly, Lys use efficiency was calculated as the sum of the Lys exported in milk true protein yield, scurf, and metabolic fecal protein divided by the Lys supply after discounting the urinary loss. Lysine supply was estimated using NRC (2001) and considering the RP-Lys bioavailability estimated in experiment 2.

Experiment 2

The companion experiment assessed the intestinal bioavailability of the RP-Lys product fed in the lactation study in comparison to free Lys using a postruminal bolus dose technique. Three lactating rumen-cannulated multiparous Holstein dairy cows averaging (mean $\pm$ SD) 192 ± 61 DIM, 724 ± 28 kg of BW, 31.4 ± 5.1 kg of milk/d were used in a 3×3 Latin square design consisting of 3 periods of 7 d each. Each period consisted of one day of postruminal bolus dosing and blood sample collection followed by 6 d of washout. Cows were housed in tiestalls, milked twice daily, and individually fed at 0600 h in amounts to ensure ad libitum intake and 10% feed refusals. Cows were fed a basal TMR throughout the entire experiment (Table 4). The diet was formulated to meet or exceed MP, dLys, and dMet requirements (NRC, 2001; Schwab et al., 2005). Milk production was recorded daily using an automatic system (Afimilk animal monitoring system, version 5.3). Cows were weighed once each period after the morning milking on the day of the postruminal bolus dosing.

Abomasal infusion lines were constructed following the procedure of Gressley et al. (2006). The treatments were bolus dosed through PVC tubing (Nalgene 980 braided clear PVC tubing; 19.05-mm i.d., 3.18-mm wall) placed into the abomasum via the reticular omasal orifice. One hundred fifty grams of RP-Lys product (39.3% of Lys, $\pm$ 0.9; NutraPass-50L, Archer Daniels Midland Co.) was bolus dosed into the abomasum; the RP-Lys was inserted into the infusion line without any pre-exposure to the rumen environment or water dilution. The positive control was 58.9 g of crystalline HCl-Lys, assumed to be 100% absorbable, diluted in 300 mL of water. The sham control was postruminal bolus dose of 360 mL of water. Following infusion, lines were flushed with additional water to total 1,500 mL of liquid infused across all treatments (1,500, 1,200, and 1,140 mL of water for RP-Lys, HCl-Lys, and water,

Table 4. Ingredient composition of the diets fed to rumen-cannulated dairy cows treated with an abomasal bolus of the rumen-protected (RP)-Lys, HCl-Lys, or water during experiment 2

Item	% of DM
Corn silage	31.7
High-moisture corn	13.3
Alfalfa haylage	9.44
Alfalfa hay	9.08
Corn grain, ground	9.05
Soybean meal	6.18
Wheat straw	5.85
Soyplus ¹	4.86
TMR blend ²	3.45
Palmitic acid ³	1.82
Calcium carbonate	1.13
Sodium bicarbonate	0.86
Blood meal	0.69
Salt	0.43
Calcium phosphate	0.35
Potassium carbonate ⁴	0.34
Omnigen AF ⁵	0.21
Magnesium oxide	0.21
Diamond V XP ⁶	0.20
Calcium sulfate	0.19
Fat yellow grease	0.19
Vitamin or trace mineral mix ⁷	0.11
Mycotoxin binder ⁸	0.06
Direct-fed microbials ⁹	0.06
Rumen-protected Met ¹⁰	0.06
Rumen-protected Lys ¹¹	0.03
Vitamin E	0.02
Rumensin ¹²	0.01
Chemical composition	
DM	45.7
CP	15.2
NDF	30.2
ADF	16.0
Crude fat	5.3
NFC	40.9
Ash	8.40
NE _L , ¹³ Mcal/kg	1.74
Ca	1.10
P	0.45
Mg	0.38
K	1.59
Na	0.50

¹Landus Cooperative.

²QLF Dairy SUGR 38 (QLF-Quality liquid feed).

³Rumen-protected palmitic acid.

⁴DCAD Plus (Church & Dwight Co. Inc.).

⁵Omnigen-AF, mixture active dried *Saccharomyces cerevisiae* and B-complex vitamins (Phibro Animal Health).

⁶Diamond V XP, *Saccharomyces cerevisiae* fermentation product (Diamond V).

⁷Purdue Dairy TMV premix: calcium, minimum 5.00%; calcium, maximum 7.00%; copper, minimum 12,000 mg/kg; selenium, minimum 260 mg/kg; zinc, minimum 54,500 mg/kg; manganese, minimum 44,500 mg/kg; iodine, minimum 440 mg/kg; cobalt, minimum 1,580 mg/kg; vitamin A, minimum 6,615,000 IU/kg; vitamin D, minimum 1,653,750 IU/kg; vitamin E, minimum 30,870 IU/kg.

⁸ETX-5 (Feedworks USA Ltd.).

⁹Select BioCycle (Agrarian Solutions).

¹⁰Smartamine M (Adisseo Inc.).

¹¹Ajipro L (Ajinomoto Co. Inc.).

¹²Rumensin (Elanco).

¹³Calculated (NRC, 2001) using treatment average DMI, weight, milk production and composition, and assayed feed composition.

respectively). The infused treatments were color coded, and the codes and group allocation were under the responsibility of the study coordinator. The codes were not revealed to other personnel involved in the data collection for the experiment.

On the infusion day, approximately 1 h before the infusion, cows were fitted with indwelling catheters (16-gauge, Abbocath-TAL catheter; Abbott Laboratories) in the left and right jugular veins for blood sample collection. The intestinal bioavailability of free Lys and the RP-Lys product was assessed using changes in plasma Lys concentrations following the postruminal bolus dose. The rise in blood concentration was used to determine the relative appearance of the RP-Lys product compared with HCl-Lys (positive control) and water (negative control).

Blood samples were collected from indwelling catheters using 12-mL syringes and placed into vacutainers containing sodium heparin (Becton, Dickinson and Co.) before (−15 min) and following the dose (30, 60, 90, 120, 180, 240, 360, and 480 min) for each treatment. The sampling interval was defined based on data presented by Fleming et al. (2019a), who observed a peak of plasma Lys concentration within 240 min after dosing when using postruminal bolus dosing of RP-Lys.

Following the sample collection, catheters were flushed and blocked with heparinized saline (2 IU/mL). Blood samples were inverted several times to ensure mixing of the anticoagulating agent and then kept on ice until centrifugation at $1,000 \times g$ for 15 min at 4°C. Centrifugation and plasma separation occurred within 30 min of each time point sample collection. Harvested plasma was stored at −80°C until analysis. Plasma samples were analyzed for AA concentration at the University of Missouri–Columbian Agricultural Experiment Station Chemical Laboratory (Columbia, MO; Deyl et al., 1986; Fekkes, 1996). Samples were analyzed once, and not in replicates.

Plasma Lys absorption was estimated from the area under curves (AUC) of plasma Lys concentration (μM) with respect to time (Chiou, 1978). The AUC was numerically estimated using the trapezoidal rule by summing the area of all the trapezoids formed between 2 time points (Cardoso et al., 2011). The relative bioavailability for RP-Lys products was determined in each experimental period as the ratio of the RP-Lys AUC over the free HCl-Lys AUC after subtracting the water treatment AUC value: Relative bioavailability = $[(AUC \text{ RP-Lys} - AUC \text{ water}) / (AUC \text{ HCl-Lys} - AUC \text{ water})]$. The relative bioavailability estimated in each period was averaged and the standard deviation calculated. The clearance rate and half-life of Lys were calculated using the kinetic analysis described previously for blood glucose (Pires et al., 2007).

Statistical Analysis

In experiment 1, weekly observations were analyzed using the MIXED procedure of SAS (ver. 9.4, SAS Institute Inc.). Cow was the experimental unit. The SCC values were log10-transformed to obtain normality. Dry matter intake, milk yield, milk composition, and components yield, feed and N efficiency, BW, BSC, and their weekly variation were analyzed with week as repeated measures. The data collected during the covariate period was used in the statistical analysis as a covariate to their respective counterparts. The statistical model for the previously described response variables included week, treatment, block, treatment $\times$ week interaction, and the covariate term. Block and block $\times$ treatment were assumed as random effects, whereas all other variables were assumed as fixed effects. The best covariance structure for repeated measures was chosen by the lowest corrected Akaike information criterion. For the plasma AA data, the model included the random effect of block, and the fixed effect of treatment. Contrast statements were used to determine the linear and quadratic effects of RP-Lys.

In experiment 2, data were analyzed using the MIXED procedure of SAS (ver. 9.4, SAS Institute Inc.). Cow within treatment was the experimental unit with 3 observations for each experimental group. Plasma AA concentration was analyzed with sampling time as a repeated measure. The statistical model included treatment, period, time, and treatment by time interaction. Cow by period was assumed as random effect, whereas all other variables were assumed as fixed effects. The best covariance structure for repeated measures was chosen by the lowest corrected Akaike information criterion. The model for milk production, DMI and Lys kinetics data accounted for the fixed effects of treatment and period, and the random effect of cow. Means were considered different when $P < 0.05$. Tukey-Kramer studentized adjustments were used for multiple comparisons. All experimental data are available upon request.

RESULTS

Experiment 1

In this experiment, we evaluated the effect of incremental amounts of a lipid-coated RP-Lys product on plasma AA concentration, milk production and composition in a diet designed to be deficient in dLys for mid-lactation dairy cows. Diet ingredients and chemical composition are presented in Tables 1 and 2. The Lys content of the RP-Lys product was (mean $\pm$ SD) 39.3 g (± 0.9) of Lys per 100 g of product. The manufactur-

Table 5. Performance data of dairy cows fed a Lys-deficient diet and either had no supplementation of a rumen-protected Lys (RP-Lys) product, or had 0.3% of diet DM or 0.6% of diet DM supplemented with a RP-Lys product (experiment 1)

Item	Treatment ¹			SEM	P-value ²	
	CON	0.3RP-Lys	0.6RP-Lys		Lin ³	Quad ⁴
BW, kg	660	665	669	3.32	0.05	0.78
BCS	3.07	3.01	2.96	0.036	0.03	0.95
DMI, kg/d	25.6	26.5	26.3	0.58	0.44	0.41
Milk, kg/d	38.4	38.0	37.2	0.72	0.24	0.87
ECM, ⁵ kg/d	34.2	33.0	33.3	0.97	0.50	0.53
Protein, %	2.99	3.02	3.06	0.034	0.20	0.96
Protein, kg/d	1.10	1.11	1.13	0.022	0.37	0.83
Fat, %	3.47	3.28	3.40	0.109	0.63	0.24
Fat, kg/d	1.29	1.21	1.24	0.053	0.46	0.44
Lactose %	4.88	4.89	4.81	0.028	0.11	0.16
Lactose, kg/d	1.80	1.81	1.79	0.045	0.84	0.75
Solids, %	12.32	12.12	12.23	0.118	0.58	0.27
Solids, kg/d	4.57	4.46	4.49	0.114	0.59	0.6
SCC, ⁶ ×1,000 cells/mL	79	52	54	0.08	0.20	0.35
MUN, mg/dL	10.18	9.38	9.48	0.332	0.14	0.26
DMI, % BW	3.9	4.0	3.9	0.08	0.81	0.28
Feed efficiency, ⁷ kg/kg	1.33	1.24	1.28	0.045	0.43	0.24
Milk N efficiency, ⁸ %	26.6	26.0	26.4	0.66	0.78	0.53
Δ BW, kg/wk	3.3	2.7	3.8	0.81	0.64	0.36
Δ BCS, points/wk	0.04	0.03	0.02	0.011	0.10	0.75

¹CON = control diet with no RP-LYS; 0.3RP-Lys = diet supplemented with 0.3% of diet DM of RP-Lys; 0.6RP-Lys = diet supplemented with 0.6% of diet DM of RP-Lys.

²No treatment × week interaction observed ($P > 0.10$).

³Linear effect.

⁴Quadratic effect.

⁵ECM (kg/d) = kg of milk × [(38.3 × % fat × 10 + 24.2 × % true protein × 10 + 16.54 × % lactose × 10 + 20.7) ÷ 3,140] (Sjaunja et al., 1991).

⁶Values presented are log₁₀ LSM (95% CI) back-transformed median.

⁷ECM/DMI.

⁸Milk N yield in true protein/N intake × 100.

er's specifications for the RP-Lys product states that 63.1% of the Lys escapes from ruminal fermentation, assessed as the proportion of the initial Lys remaining after rumen-incubation for 16 h, and is available to be absorbed in the intestine. Therefore, 24.7 g (± 1.9 SD) of Lys per 100 g of RP-Lys product were available in the intestine. Ruminal protection and absorption of the product were not tested before the execution of experiment 1. Therefore, the aforementioned manufacturer's specifications were used to determine the level of RP-Lys supplementation to the cows and the AA balance. The feeding goal for the control group was to maintain a corn-based diet to create dLys deficiency. Therefore, the control diet had 29.5% corn grain and corn by-product feeds and 42.5% corn silage on a DM basis. Diets supplemented with RP-Lys were formulated to contain similar ingredient levels as the control diet.

Dry matter intake was greater than expected for all cows in the experiment, based on the National Research Council's intake model (NRC, 2001; Table 5), although there were no differences ($P > 0.05$) in DMI (26.1 ± 0.58 kg of DM) between treatments. Because intake

was greater than expected, MP and Lys balance (NRC, 2001) was greater than expected (Table 3). Despite a greater than expected overall intake, the control diet was 10.4% (i.e., 18 g/d) deficient in dLys based on the NRC (2001) requirements, target of 6.6% of Lys as a percentage of MP.

There were no effects of RP-Lys feeding ($P > 0.05$) on milk yield, percentage of milk components, milk component yield, feed efficiency, and milk N efficiency. A linear reduction ($P = 0.03$) and tendency for linear reduction ($P = 0.05$) were observed for BCS and BW, respectively. There was no effect on plasma AA concentration (Table 6) for all essential AA ($P > 0.05$) except for His that presented a linear reduction ($P = 0.03$) as RP-Lys inclusion increased. The efficiency of Lys use decreased linearly with incremental amounts of RP-Lys ($P > 0.05$).

Experiment 2

Due to the moderate responses observed for the production study, the bioavailability of RP-Lys was

determined using a postruminal infusion model. The treatments did not alter milk production, DMI, BCS, or BW ($P > 0.05$; Supplemental Table S1; <https://www.doi.org/10.17632/9ppjcj8psw.1>, Malacco, 2022). The effects of infused Lys on plasma AA concentration are shown in Supplemental Table S2 (<https://www.doi.org/10.17632/9ppjcj8psw.1>, Malacco, 2022).

Postruminal delivery of HCl-Lys and RP-Lys products resulted in an increase in plasma Lys concentration within 30 min of dosing for HCl-Lys, and 90 min for RP-Lys that exceeded pre-infusion concentrations (Figure 1a). Plasma Lys concentration peaked (803.3 and 206.7 μM for HCl-Lys and RP-Lys, respectively)

within 120 min after the postruminal Lys bolus dose, which returned to the baseline levels within 240 min for cows receiving the RP-Lys treatment. Plasma Lys concentration was greater for HCl-Lys compared with the others within 60 min of the bolus dosing and remained elevated until 240 min. The HCl-Lys treatment resulted in greater plasma EAA and total AA concentrations when compared with the pre-infusion concentrations within 30 min of the bolus dose infusion, which was not observed for the RP-Lys and water dosing (Figure 1b and 1c, respectively). The plasma α -amino adipic acid concentration increased within 60 min after the bolus dose and remained elevated through 480 min for

Table 6. Plasma AA concentration (μM , or otherwise indicated) and Lys efficiency use of dairy cows fed a Lys-deficient diet and either had no supplementation of a rumen-protected Lys (RP-Lys) product, or had 0.3% of diet DM or 0.6% of diet DM supplemented with a RP-Lys product (experiment 1)

Item	Treatment ¹			SEM	Lin ²	Quad ³
	CON	0.3RP-Lys	0.6RP-Lys			
Arg	56.5	61.1	59.9	3.70	0.50	0.52
His	43.6	39.9	32.8	3.61	0.03	0.70
Ile	109.2	100.2	100.4	6.79	0.36	0.58
Leu	213.7	205.6	192.1	12.87	0.23	0.98
Lys	52.6	53.2	56.0	3.70	0.50	0.82
Met	46.3	48.9	51.0	3.76	0.37	0.95
Phe	46.8	46.8	43.8	1.94	0.28	0.54
Thr	85.1	82.8	82.8	5.16	0.70	0.82
Trp	25.9	24.9	24.4	1.11	0.35	0.83
Val	235.8	232.9	219.0	13.24	0.37	0.74
EAA ⁴	915.9	893.7	862.8	44.51	0.39	0.93
Ala	231.9	244.7	240.4	13.26	0.65	0.60
Asn	45.4	45.3	45.6	2.55	0.95	0.94
Asp	4.0	4.1	4.1	0.38	0.75	0.99
Gln	302.4	357.9	331.4	15.79	0.19	0.04
Glu	45.8	46.1	44.5	1.69	0.47	0.55
Gly	308.9	308.4	306.3	20.41	0.92	0.97
Pro	92.3	91.8	90.1	4.67	0.73	0.92
Ser	79.1	76.2	80.2	4.27	0.85	0.50
Tyr	56.0	55.6	57.2	3.25	0.79	0.81
NEAA ⁵	1,369.8	1,440.3	1,396.9	48.68	0.69	0.34
TAA ⁶	2,285.7	2,334.0	2,259.1	81.91	0.82	0.54
Urea	4,587.6	4,627.8	4,726.7	206.40	0.63	0.90
Aad ⁷	14.3	11.9	11.6	1.60	0.24	0.57
Cit	90.8	97.8	81.6	4.66	0.16	0.04
Orn	32.1	31.3	35.8	1.85	0.16	0.25
Hyl ⁸	1.5	1.5	1.6	0.09	0.21	0.30
1MHis ⁹	10.3	9.3	9.7	0.63	0.55	0.39
Lys efficiency of use ¹⁰	0.82	0.78	0.74	0.021	0.01	0.89

¹CON = control diet with no RP-LYS; 0.3RP-Lys = diet supplemented with 0.3% of diet DM of RP-Lys; 0.6RP-Lys = diet supplemented with 0.6% of diet DM of RP-Lys.

²Linear effect.

³Quadratic effect.

⁴Sum of EAA.

⁵Sum of NEAA.

⁶Total AA.

⁷Amino adipic acid.

⁸Hydroxylysine.

⁹1-Methyl histidine.

¹⁰Lys efficiency use calculated as described by Omphalius et al. (2020), using the RP-Lys bioavailability estimated in experiment 2, presented in arbitrary units.

Figure 1. Observed AA concentrations (μM) following a postruminal bolus infusion of the rumen-protected (RP)-Lys ($\blacktriangle$), HCl-Lys ($\blacksquare$), or water ($\bullet$) in lactating dairy cows (experiment 2). The infusion time (time 0 min) is indicated by the downward arrow. Values are LSM and SE. The effects of treatment (Trt), time, and time $\times$ Trt are indicated within each panel. The asterisk (*) denotes differences between the AA plasma concentration before the bolus dose and the plasma concentration for each time within treatment ($P < 0.05$). Letters associated with means indicate differences between treatments within each time point ($P < 0.05$).

cows receiving the HCl-Lys treatment. No effects were observed for the other treatments (Figure 1d).

Postruminal dosing with HCl-Lys resulted in a greater AUC compared with the others. There was no significant effect of form of Lys (free or RP-Lys) on plasma Lys clearance rate or half-life (Table 7). The estimated relative bioavailability of the RP-Lys products was (mean $\pm$ SD) $24.4 \pm 4.61\%$.

DISCUSSION

When evaluating the effects of incremental amounts of RP-Lys, diets were formulated to be negative in

RDP balance (-5.0%) as a strategy to change the AA profile of MP flowing from rumen to intestine. Based on the NRC (2001) model, reducing the predicted microbial protein production through limiting RDP should lead to a greater potential contribution of RUP to MP and inclusion of corn-protein sources in the diet should result in a Lys-deficient control diet. The severity of this deficiency in the feeding study, however, was less than anticipated due to greater than expected DMI and lower milk production. Compared with the DMI and milk yield values used to formulate the basal diet of the study, cows had on average 0.7 kg more DMI (25.4 vs. 26.1 kg/d) and produced 2.4 kg less milk (40.0 vs.

Table 7. Effect of abomasal bolus of the rumen-protected (RP)-Lys, HCl-Lys, or water in lactation dairy cows on plasma Lys kinetics (experiment 2)

Item	HCl-Lys	RP-Lys	Water	SEM	P-value
AUC Lys ¹	156,564 ^a	61,389 ^b	31,557 ^b	8,437.5	0.01
Lys maximum, μM	808.8 ^a	216.7 ^b	88.3 ^c	9.34	<0.01
Time maximum, min	80	100	260	58.6	0.26
Clearance rate, %/min	0.16	-0.01	-0.01	0.064	0.08
Half-life, min	546.9	853.1	-886.5	424.18	0.17

^{a-c}Means within a row with different superscripts differ ($P \leq 0.05$).

¹Area under the curve during the 480 min [$(\mu\text{M}) \cdot 480 \text{ min}$].

37.9 kg/d) throughout the study. The average DMI was 8.4% (± 1.4 SD) greater than predicted by NRC (2001) model with negative RDP balance and without differences between treatments. The greater DMI shows that the negative RDP balance probably did not compromise ruminal fiber digestibility and, consequently, intake (Allen, 2000). The lack of RP-Lys dose effect on DMI is in line with data from other studies that evaluate RP-Lys sources (Giallongo et al., 2016; Lee et al., 2019; Morris and Kononoff, 2020).

Cows in the control group were 10.4% deficient in Lys (i.e., -18 g of dLys). The RP-Lys supplementation doses were calculated using the manufacturer's specification of 39.3% of Lys and 63.1% of bioavailability that would result in observed dLys balances of -18 , 4, and 21 g of Lys for CON, 0.3RP-Lys and 0.6RP-Lys, respectively. However, bioavailability of the RP-Lys product estimated in the present study (experiment 2) was lower than specified by manufacturers (24.4%), resulting in a lower-than-expected supply of dLys (8 and 15 g of dLys for 0.3RP-Lys and 0.6RP-Lys, respectively), which was not sufficient to meet the Lys requirements resulting in deficiencies of -9 and -3 g of dLys for 0.3RP-Lys and 0.6RP-Lys, respectively. Herein, the discussion will be based on the bioavailability estimated in experiment 2.

We hypothesized that supplementing Lys in a Lys-deficient diet would increase milk protein concentration and yield, which would be an expected outcome when balancing AA in diets of high-producing dairy cows (NRC, 2001). However, milk protein concentration and yield were unchanged. Additionally, milk yield, ECM, fat, lactose concentration and yield did not change due to the incremental levels of RP-Lys supplementation. Lysine is recognized as the first limiting AA for milk protein synthesis in early lactation dairy cows and co-limiting with Met in mid-lactating dairy cows (Schwab et al., 1992). Responses in milk production and protein yield and concentration to Lys supplementation have been reported when infused (Schwab et al., 1976; Rulquin et al., 1993) or fed in a RP form (Giallongo et al., 2016; Bailey et al., 2019; Lobos et al., 2021) for cows postpeak of lactation. Bailey et al. (2019) reported a linear response in milk yield due to supplementation with various levels of the RP-Lys prototype. However, the milk yield response is variable, and several other studies have reported no effects to RP-Lys supplementation in agreement with our observations (Arriola Apelo et al., 2014; Giallongo et al., 2016; Morris and Kononoff, 2020). In addition, lack of responses in milk production, milk protein production, and changes in plasma Lys due to RP-Lys supplementation are also reported in the literature corroborating our results (Arriola Apelo et al., 2014; Bernard et al., 2014; Morris and Kononoff, 2020). Giallongo et al. (2016) observed

no effect on milk yield, fat yield, lactose yield, protein yield, fat concentration, and lactose concentration but observed changes in milk true protein concentration when supplementing RP-Lys in a MP and Lys-deficient diet. The negative MP balance observed in the above-mentioned study, despite the less severe Lys deficiency than observed in the present experiment, may have exacerbated the negative effect of AA deficiency to the mammary gland, thus, leading to a significant response in milk protein composition due to Lys supplementation in a RP form.

In the present study, the basal diet was formulated to meet or exceed the dMet requirements of 2.2% of MP recommended on NRC (2001). Thus, this AA was not expected to limit possible responses to an increasing level of dLys. However, other factors than adequate dMet diets could have impaired the response, including but not limited to the stage of lactation, other colimiting AA, or the dLys supply. Cows in the study were in mid-lactation and less prone to the limitations in milk and protein yield due to the observed mild Lys deficiency in the diets.

In the current experiment, there were no observed effects of level of RP-Lys on MUN, although the values were similar or lower than the values observed in other studies (Swanepoel et al., 2010; Mullins et al., 2013; Weiss, 2019) suggesting N supply was adequate but not excessive or severely imbalanced. There was no effect of treatment on efficiency parameters. However, values of feed efficiency and N use efficiency obtained were similar to those observed by Huhtanen and Hristov (2009).

Plasma AA concentrations reflect the balance between their supply and utilization, and therefore, an increase in plasma Lys was expected after the supplementation exceeded requirements. However, as mentioned before in the discussion, the bioavailability of RP-Lys used in this study was lower than anticipated and only a numerical increase in plasma Lys concentration was observed, confirming that the supplemented RP-Lys was not adequate to meet Lys requirement. Previous studies with RP-Lys using greater inclusions (17.5 to 31 g of dLys/d) than used in this study have shown no response in plasma Lys concentration (Fehlberg et al., 2020; Lobos et al., 2021; McLain et al., 2021). Several factors can affect the plasma AA concentration, and increasing supplies of Lys can modify the overall utilization of AA, reducing AA use efficiency for milk protein synthesis. For instance, Lys uptake by the mammary gland exceeds the secretion into milk protein, and this excessive Lys removal by the mammary gland increases with increased Lys supply (Lapierre et al., 2012). Although lower than the mammary gland, the liver contributes a minor proportion of the Lys removal, and it appears to mainly be a mass action function of total

liver inflow with an increase in the expected removal with increased Lys supply (Fleming et al., 2019b). The lack of response in plasma Lys concentration due to supplementation, even when Lys is fed in excess, could be related to this higher removal rate and reduced efficiency by the mammary gland and liver.

There was no effect of treatment on plasma EAA concentration except for His. Plasma His concentration decreases linearly ($P < 0.05$) by RP-Lys feeding. The prediction of plasma His concentration based on the duodenal His flow as percentage of MP for the cows in this present study, using the equation presented by Patton et al. (2015), yielded plasma His concentrations of 41.3, 40.9, and 40.7 μM for CON, 0.3RP-Lys, and 0.6RP-Lys, respectively. Although a numerical reduction across treatments was observed, the estimate for the highest level is 35% greater than the 30 μM observed for 0.6RP-Lys in the present trial. Previous studies suggest that Lys supplied in excess of tissue requirements could result in an AA imbalance that would negatively affect animal production and performance (Robinson et al., 2000).

Studies have shown that His deficiency is more evident in diets with lower RUP supply, commonly observed in grass-silage-based or MP negative diets, but His deficiency can be observed impairing milk and milk protein yield even in cows fed with MP-adequate diets (Morris and Kononoff, 2020). Histidine requirement was proposed by Lee et al. (2012) as 2.2% MP requirements, and in this present study, using those proposed His requirements and the dHis flow estimated using the NRC (2001) resulted in a His balance of 0 g across all treatments. Weekes et al. (2006) evaluating large AA imbalances and deficiencies using intravenous infusion of a mixture of AA (milk AA profile) and the same mixture lacking Lys, Met, or His, observed a severe reduction in plasma His concentration compared with the negative control, without any changes in milk protein production when His was removed from the mixture. The authors suggested that reduction in His not accompanied by changes in milk production or milk components yield could be explained by their use for other tissues in the body. In the present study, we speculate that the imbalance created by supplementing Met and incremental levels of Lys without changing His concentration could have created AA imbalance directing His use for another tissue.

The calculated efficiency of Lys utilization reduced linearly with the increased dLys supply, which was expected with the similar milk protein yield observed regardless of increasing dietary supply of Lys. Given the lack of effects of RP-Lys supplementation on milk production and composition and the lack of negative consequences of RP-Lys supplementation on feed intake

or BW changes, but the associated changes in plasma His with RP-Lys feeding, we can infer that feeding RP-Lys has a physiological effect on mid-lactation dairy cows. Presently, however, the targets for increased dLys supply (i.e., increase plasma Lys concentration and cows' productivity performance, remain elusive).

A primary goal in feeding RP AA is to correct deficiencies or imbalance of the specific AA through the release of these AA in the intestine. Therefore, assessing the changes in plasma AA concentration in response to acute changes in postruminal AA supply is a strategy that has been used to test the ability of RP-Lys products to deliver AA that can be absorbed in the small intestine (Blum et al., 1999; Whitehouse et al., 2017). This approach assumes that a pulse dose of AA delivered postruminally can be detected as a change in plasma AA concentration over time. Furthermore, the area under the curve for Lys concentration by time provides an assessment of an RP-Lys product's relative capacity to deliver Lys compared with free Lys (HCl-Lys).

Although the postruminal dosing protocol is a valuable technique to examine the effects of postruminal availability of nutrients, a drawback of the method when used for RP-Lys is the lack of previous exposure of the test material to the feed, mastication, and the rumen environment, and a combination of these events. The bioavailability of RP-Lys can be overestimated or underestimated when not subject to ruminal incubation. Studies have shown that losses in Lys in the rumen environment are related to the rumen conditions and not only to a washout effect (Block and Jenkins, 1994). If AA remains on the surface of the RP-Lys product during the production process, they may be solubilized by ruminal microorganisms compromising the AA core of the product. However, when coated with saturated fat in a prilled form, as the RP-Lys used in this present study, they are subject to minimal rumen effects, and the increased melting points of the saturated fat also prevent microbial penetration into the prill matrix (Ferguson et al., 1990; Jenkins and Jenny, 1992). The retention time is also associated with the failure of the AA delivery to the small intestine. Longer retention time can increase the exposure to ruminal microorganisms, thereby increasing the chance of AA solubilization and degradation (Block and Jenkins, 1994). However, the rumen abrasion and mechanical force may positively affect the prill's intestinal digestibility. The rumen action could create an additional surface area without releasing the AA, increasing the emulsification of the coating fat then increasing the AA release and absorption in the intestine (Fleming et al., 2019a).

Plasma AA concentrations reflect the balance between AA supply and utilization. In experiment 2, Lys

provided by the RP-Lys product increased Lys plasma concentration after a bolus dose compared with pre-infusion levels and the sham control. This observation showed the possibility of manipulating the ratio of Lys related to other EAA while using the RP-Lys product aimed to achieve the desirable EAA profile for milk protein synthesis and improve N use efficiency (Arriola Apelo et al., 2014). The increase in the EAA concentration after a bolus dose of HCl-Lys treatment was expected due to an increase in intestinal supply of Lys with bolus dosing of Lys. An increase in plasma concentration of α -amino adipic acid was expected when Lys was bolus dosed, on a free form as HCl-Lys or RP form, and absorbed. However, increases in concentration were only observed when HCl-Lys was infused and although greater than the postinfusion value, the plasma concentration of α -amino adipic acid did not differ among treatments. Changes in α -amino adipic acid were expected as it is the primary breakdown product of Lys in its major degradation pathway, and effects of prolonged Lys infusion to increase circulating α -amino adipic acid concentrations were previously reported (Tucker et al., 2017). Moreover, Lys supply has been shown to control Lys metabolism in liver, albeit a minor site of Lys catabolism (Fleming et al., 2019a).

The results of the postruminal bolus dose infusion show that the RP-Lys source was able to deliver Lys for intestinal absorption. This statement can be made based on the difference between plasma concentrations before the bolus dose and when comparing with plasma concentrations of the sham control. Several key factors suggest that the bioavailability of the RP-Lys source compared with free Lys should be interpreted with caution. Intestinal availability of AA is related to the ruminal protection providing that the protective coating can release the AA in the abomasum and duodenum. In cases where there is inconsistency in the postruminal degradation of the protective coating, there is a failure in AA delivery for absorption (Koenig and Rode, 2001; Robinson et al., 2010).

Furthermore, bolus dosing RP-Lys product resulted in 75 g of hydrogenated soybean oil (87.8% of C18:0 fatty acid) dosed into the small intestines. Consequently, the bolus dose of the product may have exceeded the intestine's capacity to digest the lipid coating and impaired the release of the AA (Wu et al., 2012; Boerman et al., 2015). Further work is needed to assess dosing time and amount to more fully evaluate the utility of postruminal dosing in the assessment of the biological availability of RP AA. Additionally, the bioavailability in this study was assessed without prior exposure of the RP-Lys product to the basal diet, feeding process, act of eating, and ruminal digestion; thus, the calculated bioavailability could vary depending on the effects of

each one of those factors and the interaction among them.

Although supplemental RP-Lys increased the estimated digestible Lys supply, the results observed did not support the hypothesis that increasing the Lys supply would increase plasma Lys concentration, milk production, and milk composition of cows when feeding a Lys-deficient diet. Multiple AA can co-limit with Lys when corn-based diets are fed for lactating dairy cows (perhaps His in this evaluation), which could prevent the potential response in milk yield and milk components expected from the increased supply of dLys.

CONCLUSIONS

The results of this study indicated that the RPLys product was able to deliver Lys and be absorbed in the small intestine based on the difference in the pre- and postinfusion plasma Lys concentration with an estimated bioavailability of 24.4%. The lack of expected positive responses to the RP-Lys supplementation in the lactation study must be considered in light of the lower-than-expected Lys supply from RP-Lys based on bioavailability estimated in this study. Moreover, the lack of measuring the effects of prefeeding practices and all digestion processes could have interfered in the AA bioavailability evaluation and needs to be investigated in future studies.

ACKNOWLEDGMENTS

The study was supported by funds from Archer Daniels Midland Company (Decatur, IL). Funding to support V. M. R. Malacco was from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–Brasil (CAPES)–Finance Code 001. The authors have not stated any conflicts of interest.

REFERENCES

- Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J. Dairy Sci.* 83:1598–1624. [https://doi.org/10.3168/jds.S0022-0302\(00\)75030-2](https://doi.org/10.3168/jds.S0022-0302(00)75030-2).
- Alves, D. 2004. Nutrição aminoacídica de bovinos. *Curr. Agric. Sci. Technol.* 10:265–271.
- AOAC International. 2000. *Official Methods of Analysis*. 17th ed. AOAC International.
- AOAC International. 2006. *Official Methods of Analysis*. 18th ed. AOAC International.
- ARC (Agricultural Research Council). 1981. *The Nutrient Requirements of Pigs*. Commonwealth Agricultural Bureaux.
- Arriola Apelo, S. I., A. L. Bell, K. Estes, J. Ropelewski, M. J. de Veth, and M. D. Hanigan. 2014. Effects of reduced dietary protein and supplemental rumen-protected essential amino acids on the nitrogen efficiency of dairy cows. *J. Dairy Sci.* 97:5688–5699. <https://doi.org/10.3168/jds.2013-7833>.
- Bailey, H. R., J. D. Kaufman, K. A. Estes, C. A. Zimmerman, B. A. Barton, and A. G. Rius. 2019. Rumen-protected lysine supplement

- tation increased milk production in dairy cows fed a lysine-deficient diet. *Appl. Anim. Sci.* 35:482–490. <https://doi.org/10.15232/aas.2019-01885>.
- Behan, A. A., T. C. Loh, S. Fakurazi, U. Kaka, A. Kaka, and A. A. Samsudin. 2019. Effects of supplementation of rumen protected fats on rumen ecology and digestibility of nutrients in sheep. *Animals (Basel)* 9:400. <https://doi.org/10.3390/ani9070400>.
- Bernard, J. K., P. T. Chandler, C. J. Sniffen, and W. Chalupa. 2014. Response of cows to rumen-protected lysine after peak lactation. *Prof. Anim. Sci.* 30:407–412. <https://doi.org/10.15232/pas.2014-01307>.
- Berthiaume, R., H. Lapierre, M. Stevenson, N. Coté, and B. W. McBride. 2000. Comparison of the in situ and in vivo intestinal disappearance of ruminally protected methionine. *J. Dairy Sci.* 83:2049–2056. [https://doi.org/10.3168/jds.S0022-0302\(00\)75086-7](https://doi.org/10.3168/jds.S0022-0302(00)75086-7).
- Block, S. M., and T. C. Jenkins. 1994. The use of prilled fat to coat and protect amino acids from ruminal degradation. *J. Sci. Food Agric.* 65:441–447. <https://doi.org/10.1002/jsfa.2740650412>.
- Blum, J. W., R. M. Bruckmaier, and F. Jans. 1999. Rumen-protected methionine fed to dairy cows: Bioavailability and effects on plasma amino acid pattern and plasma metabolite and insulin concentrations. *J. Dairy Sci.* 82:1991–1998. [https://doi.org/10.3168/jds.S0022-0302\(99\)75435-4](https://doi.org/10.3168/jds.S0022-0302(99)75435-4).
- Boerman, J. P., J. L. Firkins, N. R. St-Pierre, and A. L. Lock. 2015. Intestinal digestibility of long-chain fatty acids in lactating dairy cows: A meta-analysis and meta-regression. *J. Dairy Sci.* 98:8889–8903. <https://doi.org/10.3168/jds.2015-9592>.
- Bröer, S. 2008. Amino acid transport across mammalian intestinal and renal epithelia. *Physiol. Rev.* 88:249–286. <https://doi.org/10.1152/physrev.00018.2006>.
- Cardoso, F. C., W. Sears, S. J. LeBlanc, and J. K. Drackley. 2011. Technical note: Comparison of 3 methods for analyzing areas under the curve for glucose and nonesterified fatty acids concentrations following epinephrine challenge in dairy cows. *J. Dairy Sci.* 94:6111–6115. <https://doi.org/10.3168/jds.2011-4627>.
- Castillo, A. R., E. Kebreab, D. E. Beever, and J. France. 2000. A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. *J. Anim. Feed Sci.* 9:1–32. <https://doi.org/10.22358/jafs/68025/2000>.
- Chiou, W. L. 1978. Critical evaluation of the potential error in pharmacokinetic studies of using the linear trapezoidal rule method for the calculation of the area under the plasma level-time curve. *J. Pharmacokinet. Biopharm.* 6:539–546. <https://doi.org/10.1007/BF01062108>.
- Coleman, C. E., and B. A. Larkins. 1999. The prolamins of maize. Pages 109–139 in *Seed Proteins*. P. R. Shewry and R. Casey, ed. Taylor & Francis Publishers.
- Danfær, A., and J. A. Fernández. 1999. Developments in the prediction of nutrient availability in pigs: A review. *Acta Agric. Scand. Sect. Ann. Anim. Sci.* 49:73–82.
- Deyl, Z., J. Hyaneek, and M. Horakova. 1986. Profiling of amino acids in body fluids and tissues by means of liquid chromatography. *J. Chromatogr.* 379:177–250. [https://doi.org/10.1016/S0378-4347\(00\)80685-4](https://doi.org/10.1016/S0378-4347(00)80685-4).
- Fehlberg, L. K., A. R. Guadagnin, B. L. Thomas, Y. Sugimoto, I. Shinzato, and F. C. Cardoso. 2020. Feeding rumen-protected lysine prepartum increases energy-corrected milk and milk component yields in Holstein cows during early lactation. *J. Dairy Sci.* 103:11386–11400. <https://doi.org/10.3168/jds.2020-18542>.
- Fekkes, D. 1996. State-of-the-art of high-performance liquid chromatographic analysis of amino acids in physiological samples. *J. Chromatogr. B Biomed. Appl.* 682:3–22. [https://doi.org/10.1016/0378-4347\(96\)00057-6](https://doi.org/10.1016/0378-4347(96)00057-6).
- Ferguson, J. D., D. Sklan, W. V. Chalupa, and D. S. Kronfeld. 1990. Effects of hard fats on in vitro and in vivo rumen fermentation, milk production, and reproduction in dairy cows. *J. Dairy Sci.* 73:2864–2879. [https://doi.org/10.3168/jds.S0022-0302\(90\)78974-6](https://doi.org/10.3168/jds.S0022-0302(90)78974-6).
- Fleming, A. J., K. A. Estes, H. Choi, B. A. Barton, C. A. Zimmerman, and M. D. Hanigan. 2019a. Assessing bioavailability of ruminally protected methionine and lysine prototypes. *J. Dairy Sci.* 102:4014–4024. <https://doi.org/10.3168/jds.2018-14667>.
- Fleming, A. J., H. Lapierre, R. Martineau, R. R. White, and M. D. Hanigan. 2019b. Modeling portal-drained viscera and liver fluxes of essential amino acids in dairy cows. *J. Dairy Sci.* 102:10964–10982. <https://doi.org/10.3168/jds.2019-16302>.
- 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>.
- Godden, S. M., K. D. Lissimore, D. F. Kelton, K. E. Leslie, J. S. Walton, and J. H. Lumsden. 2001. Relationships between milk urea concentrations and nutritional management, production, and economic variables in Ontario dairy herds. *J. Dairy Sci.* 84:1128–1139. [https://doi.org/10.3168/jds.S0022-0302\(01\)74573-0](https://doi.org/10.3168/jds.S0022-0302(01)74573-0).
- Gressley, T. F., S. M. Reynal, J. J. Olmos Colmenero, G. A. Broderick, and L. E. Armentano. 2006. Technical note: Development of a tool to insert abomasal infusion lines into dairy cows. *J. Dairy Sci.* 89:3965–3967. [https://doi.org/10.3168/jds.S0022-0302\(06\)72438-9](https://doi.org/10.3168/jds.S0022-0302(06)72438-9).
- Harmon, D.L., and K.C. Swanson. 2020. Review: Nutritional regulation of intestinal starch and protein assimilation in ruminants. *Animal* 14(S1):s17–s28.
- Huhtanen, P., and A. N. Hristov. 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. *J. Dairy Sci.* 92:3222–3232. <https://doi.org/10.3168/jds.2008-1352>.
- Jenkins, T. C., and B. F. Jenny. 1992. Nutrient digestion and lactation performance of dairy cows fed combinations of prilled fat and canola oil. *J. Dairy Sci.* 75:796–803. [https://doi.org/10.3168/jds.S0022-0302\(92\)77818-7](https://doi.org/10.3168/jds.S0022-0302(92)77818-7).
- Koenig, K. M., and L. M. Rode. 2001. Ruminal degradability, intestinal disappearance, and plasma methionine response of rumen-protected methionine in dairy cows. *J. Dairy Sci.* 84:1480–1487. [https://doi.org/10.3168/jds.S0022-0302\(01\)70181-6](https://doi.org/10.3168/jds.S0022-0302(01)70181-6).
- Lapierre, H., G. E. Lobley, L. Doepel, G. Raggio, H. Rulquin, and S. Lemosquet. 2012. Triennial lactation symposium: Mammary metabolism of amino acids in dairy cows. *J. Anim. Sci.* 90:1708–1721. <https://doi.org/10.2527/jas.2011-4645>.
- Lee, C., A. N. Hristov, T. W. Cassidy, K. S. Heyler, H. Lapierre, G. A. Varga, M. J. De Veth, R. A. Patton, and C. Parys. 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. *J. Dairy Sci.* 95:6042–6056. <https://doi.org/10.3168/jds.2012-5581>.
- 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>.
- Lobos, N. E., M. A. Wattiaux, and G. A. Broderick. 2021. Effect of rumen-protected lysine supplementation of diets based on corn protein fed to lactating dairy cows. *J. Dairy Sci.* 104:6620–6632. <https://doi.org/10.3168/jds.2020-19835>.
- Malacco, V. 2022. Effects of increased doses of lysine in a rumen-protected form on plasma amino acid concentration and lactational performance of dairy cows fed a lysine-deficient diet. *Mendeley Data*, V1. <https://doi.org/10.17632/9ppjcj8psw.1>.
- McLain, K. A., D. L. Morris, and P. J. Kononoff. 2021. Effect of feeding hydrolyzed feather meal and rumen-protected lysine on milk protein and energy utilization in late-lactation Jersey cows. *J. Dairy Sci.* 104:8708–8720. <https://doi.org/10.3168/jds.2020-19657>.
- Morris, D. L., and P. J. Kononoff. 2020. Effects of rumen-protected lysine and histidine on milk production and energy and nitrogen utilization in diets containing hydrolyzed feather meal fed to lactating Jersey cows. *J. Dairy Sci.* 103:7110–7123. <https://doi.org/10.3168/jds.2020-18368>.
- Mullins, C. R., D. Weber, E. Block, J. F. Smith, M. J. Brouk, and B. J. Bradford. 2013. Short communication: Supplementing lysine and methionine in a lactation diet containing a high concentration

- of wet corn gluten feed did not alter milk protein yield. *J. Dairy Sci.* 96:5300–5305. <https://doi.org/10.3168/jds.2013-6755>.
- NRC (National Research Council). 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. National Academies Press.
- Omphalius, C., S. Lemosquet, D. R. Ouellet, L. Bahloul, and H. Lapiere. 2020. Postruminal infusions of amino acids or glucose affect metabolisms of splanchnic, mammary, and other peripheral tissues and drive amino acid use in dairy cows. *J. Dairy Sci.* 103:2233–2254. <https://doi.org/10.3168/jds.2019-17249>.
- Patton, R. A., A. N. Hristov, C. Parys, and H. Lapierre. 2015. Relationships between circulating plasma concentrations and duodenal flows of essential amino acids in lactating dairy cows. *J. Dairy Sci.* 98:4707–4734. <https://doi.org/10.3168/jds.2014-9000>.
- Pires, J. A. A., A. H. Souza, and R. R. Grummer. 2007. Induction of hyperlipidemia by intravenous infusion of tallow emulsion causes insulin resistance in Holstein cows. *J. Dairy Sci.* 90:2735–2744. <https://doi.org/10.3168/jds.2006-759>.
- Robinson, P. H., W. Chalupa, C. J. Sniffen, W. E. Julien, H. Sato, T. Fujieda, T. Ueda, and H. Suzuki. 2000. Influence of abomasal infusion of high levels of lysine or methionine, or both, on ruminal fermentation, eating behavior, and performance of lactating dairy cows. *J. Anim. Sci.* 78:1067–1077. <https://doi.org/10.2527/2000.7841067x>.
- Robinson, P. H., N. Swanepoel, and E. Evans. 2010. Effects of feeding a ruminally protected lysine product, with or without isoleucine, valine and histidine, to lactating dairy cows on their productive performance and plasma amino acid profiles. *Anim. Feed Sci. Technol.* 161:75–84. <https://doi.org/10.1016/j.anifeedsci.2010.07.017>.
- Rulquin, H., P. M. Pisulewski, R. Vrit, and J. Guinard. 1993. Milk production and composition as a function of postruminal lysine and methionine supply: A nutrient-response approach. *Livest. Prod. Sci.* 37:69–90. [https://doi.org/10.1016/0301-6226\(93\)90065-P](https://doi.org/10.1016/0301-6226(93)90065-P).
- Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and M. M. A. Mesbah. 1992. Amino acid limitation and flow to duodenum at four stages of lactation. 1. Sequence of lysine and methionine limitation. *J. Dairy Sci.* 75:3486–3502. [https://doi.org/10.3168/jds.S0022-0302\(92\)78125-9](https://doi.org/10.3168/jds.S0022-0302(92)78125-9).
- Schwab, C. G., P. Huhtanen, C. W. Hunt, and T. Hvelplund. 2005. *Nitrogen Requirements of Cattle*. CAB Int.
- Schwab, C. G., L. D. Satter, and A. B. Clay. 1976. Response of lactating dairy cows to abomasal infusion of amino acids. *J. Dairy Sci.* 59:1254–1270. [https://doi.org/10.3168/jds.S0022-0302\(76\)84354-8](https://doi.org/10.3168/jds.S0022-0302(76)84354-8).
- Sjaunja, L. O., L. Baevre, L. Junkkarinen, J. Pedersen, and J. Setl. 1991. A Nordic proposal for an energy corrected milk (ECM) formula. Pages 156–157 in *Proceedings of the 27nd Biennial Session of the Committee for Animal Recording (ICAR)*. Paris, France. Centre for Agricultural Publishing and Documentation (Pudoc).
- 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).
- 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>.
- Tucker, H. A., M. D. Hanigan, J. Escobar, P. H. Doane, and S. S. Donkin. 2017. Hepatic expression of aminoadipate semialdehyde synthase is unchanged by postruminal lysine supply in lactating dairy cows. *J. Dairy Sci.* 100:1009–1018. <https://doi.org/10.3168/jds.2016-10972>.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2).
- Weekes, T. L., P. H. Luimes, and J. P. Cant. 2006. Responses to amino acid imbalances and deficiencies in lactating dairy cows. *J. Dairy Sci.* 89:2177–2187. [https://doi.org/10.3168/jds.S0022-0302\(06\)72288-3](https://doi.org/10.3168/jds.S0022-0302(06)72288-3).
- 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>.
- Whitehouse, N. L., C. G. Schwab, and A. F. Brito. 2017. The plasma free amino acid dose-response technique: A proposed methodology for determining lysine relative bioavailability of rumen-protected lysine supplements. *J. Dairy Sci.* 100:9585–9601. <https://doi.org/10.3168/jds.2017-12695>.
- Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. *J. Dairy Sci.* 65:495–501. [https://doi.org/10.3168/jds.S0022-0302\(82\)82223-6](https://doi.org/10.3168/jds.S0022-0302(82)82223-6).
- Wu, Z., J. K. Bernard, R. B. Eggleston, and T. C. Jenkins. 2012. Ruminant escape and intestinal digestibility of ruminally protected lysine supplements differing in oleic acid and lysine concentrations. *J. Dairy Sci.* 95:2680–2684. <https://doi.org/10.3168/jds.2011-5203>.