Lysine bioavailability among 2 lipid-coated lysine products after exposure to silage[1]

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ABSTRACT: We conducted 2 experiments to determine lysine bioavailability from 2 lipid-coated lysine products. In an in vitro experiment we mixed each lipid-coated lysine product with either alfalfa- or corn-silage at different amounts of acidity. Scanning electron micrographs indicated that surface structure of each lipid-coated lysine particle was eroded after mixing with silage. Additionally, visual evaluation of scanning electron micrographs suggested that peripheral surface abrasion of lipid-coated lysine may be greater when lipid-coated lysine was mixed with alfalfa silage in comparison to corn silage. In a corresponding experiment, in vivo measures of lysine bioavailability to sheep from 2 lipid-coated lysine products and lysine-HCl were determined after mixing in corn silage. Plasma lysine concentrations increased linearly ($P < 0.01$) in response to abomasal lysine infusion indicating that our model was sensitive to increases in metabolizable lysine flow. Bioavailability of each lipid-coated lysine source and dietary lysine-HCl were calculated to be 23, 15, and 18%, respectively. Even though each dietary source of lysine increased plasma lysine, rates of increases in plasma lysine from one lipid-coated lysine source (linear; $P = 0.20$) and lysine-HCl (linear; $P = 0.11$) were not different from plasma lysine levels supported by diet alone. However, the rate of plasma lysine increase in response to lysine from the other lipid-coated lysine source was greater ($P = 0.04$) than plasma lysine from feed alone. Nonetheless, the rate of plasma lysine increase in response to lipid-coated lysine did not differ ($P \geq 0.70$) from the rate of plasma lysine increase from lysine-HCl. Clearly, methods of manufacture, together with physical and chemical characteristics of diet, can impact amounts of metabolizable lysine provided from lipid-coated lysine products. Direct measures of lysine bioavailability from lipid-coated lysine products after mixing with diets should be based on measurements with the products treated similarly to the method of feeding.

Key words: amino acid, bioavailability, cattle, lysine, silage

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INTRODUCTION

Protein synthesis and milk protein production among lactating cows fed corn-based diets is often first-limited by metabolizable lysine (NRC, 2001). Therefore, efficiency of N used for productive purposes (i.e., milk N and N retained) is increased when lactating cows are fed diets with greater amounts of metabolizable lysine. Specifically, greater metabolizable lysine can increase milk protein content and yield (Clark, 1975; NRC, 2001; Paz and Kononoff, 2014) and milk protein secretions. Several authors have also reported that greater amounts of metabolizable lysine can also improve milk fat content and secretions (Bremmer et al., 1997; Xu et al., 1998). Most feeds commonly used to increase flow of metabolizable protein to cows do not perfectly complement metabolizable proteins from corn. Thus, supplementation of complex proteins to improve efficiency of N use in cows is limited in comparison to supplementation of individual amino acids to meet limits in metabolizable amino acids. Unfortunately,

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most free amino acids are rapidly degraded by ruminal microbiota (Lewis and Emery, 1962; Chalupa, 1975) and supplementation of crystalline amino acids provides only small increases in metabolizable amino acid supply (Mangan, 1972). Many technologies have been developed to increase metabolizable amino acid flows from crystalline amino acids to ruminants (Wu and Papas, 1997). Hydroxymethyl analogs (Elwakeel et al., 2012), acid degradable polymeric coatings (Titgemeyer et al., 1988; Klemesrud et al., 2000) and lipid coatings (Block and Jenkins, 1994) have been suggested as methods to increase metabolizable lysine supplies from crystalline lysine. However, many diets fed to cows limited by metabolizable lysine contain appreciable amounts of acidic ingredients (e.g., corn silage, alfalfa silage) that may diminish effectiveness of technologies sensitive to pH, and mechanical forces associated with mixing may disturb capsule coatings. Most feed technologies currently used to augment metabolizable lysine supply from crystalline lysine utilize lipid coating. Yet, few data are available on amounts of metabolizable lysine provided from lipid-coated lysine. Most evaluations of lipid-coated lysine are limited to measures of lysine loss from lipid-coated lysine after ruminal incubation or performance responses. Several reports suggest that appreciable amounts of lysine are lost from lipid-coated lysine after ruminal incubation and Wu et al. (2012) calculated that less than half of lipid-coated lysine was able to disappear from mobile bags placed in the intestine. Direct measures of metabolizable lysine to ruminants allows producers and nutritionists to appropriately value supplemental lysine sources when formulating diets designed to meet metabolizable lysine requirements. Therefore, our objective in this work was to directly measure amounts of metabolizable lysine provided to ruminants from 2 lipid-coated lysine products after exposure to silage.

MATERIALS AND METHODS

All procedures involving the use of animals were approved by the South Dakota State University Institutional Animal Care and Use Committee.

Experiment 1

In a preliminary experiment, we evaluated effects of mixing 2 lipid-coated lysine products with alfalfa- or corn-silage at 2 different amounts of acidity. Alfalfa and corn silage were prepared by chopping alfalfa (50% DM; average particle size = 0.81 cm) or whole corn plants (46% DM; average particle size = 0.79 cm). Chopped alfalfa (0.7 ± 0.05 kg/L) and whole corn plants (0.6 ± 0.05 kg/L) were each packed separately into 4 miniature silos (121 L; 2 miniature silos for each silage type) and ensiled for 169 and 154 d, respectively. Alfalfa (pH = 4.4 ± 0.1) and corn silage (pH = 3.7 ± 0.1) from each miniature silo were composited by silage type after ensiling.

Acidity of silage was modified prior to incubation of lipid-coated lysine to evaluate effects of acidity and silage type on surface structure of lipid-coated lysine. The pH in an aliquot (9.5 kg DM) of alfalfa silage was increased to 6.8 after initial measures of acidity by mixing (model 2030, Marion Mixer) with 2.8 kg of 10% (wt/wt) NaOH for 5 min. Similarly, pH in 1 aliquot of corn silage (11 kg DM) was adjusted to be similar to alfalfa silage (pH = 4.6) by addition of 1.55 kg of 10% (wt/wt) NaOH, and acidity in another aliquot (11 kg DM) of corn silage was modified by mixing 3.15 kg of 10% (wt/wt) NaOH to achieve a pH (pH = 6.9) similar to the aliquot of alfalfa silage with added NaOH.

Samples (4 g) of 2 lipid-coated lysine products were each placed in heat sealed polyethylene bags (10 × 20 cm, pore size = 50 μm; Dacron, Ankom Technology, Fairport, NY) and hand-mixed with each silage immediately after modification of silage acidity. One lipid-coated lysine product (EB; LysiPEARL, Kemin Industries, Des Moines, IA) was manufactured by extrusion of lysine-HCl and lipid into small particles and contained 47.5% lysine-HCl and 52.5% lipid. Similarly, the other lipid-coated lysine product (EC; USA Lysine, Kemin Industries) was also manufactured by extrusion of lysine-HCl and lipid into small particles and subsequently the lysine-lipid particles were encapsulated with lipid; EC contained 65% lysine-HCl and 35% lipid. After samples of lipid-coated lysine were incubated in silage for 24 h polyethylene bags were removed and immediately rinsed with 5 L of cold tap water per side over a 40 μm screen. After rinsing, samples were frozen (–20°C) and lyophilized prior to removal from polyethylene bags. Effects of acidity and silage type on surface structure of each lipid-coated lysine was imaged by a scanning electron micrograph (Hitachi S-3400 N) after coating (10 nm) replicate subsamples of each lipid-coated lysine with Au and 2 scanning electron micrographs from each replicate were captured. Representative scanning electron micrographs are presented in Figs. 1, 2, and 3, respectively. All captured scanning electron micrographs are available as supplementary material (Supplemental Figures S1 thrugh S12; see online version of article to access the material). Samples of lipid-coated lysine not incubated with silage were imaged to allow visualization of lipid-coated lysine surface structure before incubation with silage and prior to rinsing freezing and lyophilizing. Additionally, samples (4 g) of each lipid-coated lysine placed in polyethylene bags rinsed with 5 L of cold tap water per side over a 40 μm screen, frozen (–20 °C) and lyophilized but not incubated with silage were imaged

Table 1. Composition of diet fed to ewes in Exp. 2

Item	% of diet dry matter
Ingredient composition, % dry matter	
Corn silage	50.00
Dry-rolled corn	42.81
Cane molasses	2.50
Fishmeal	2.00
Limestone	1.00
Urea	0.66
Salt	0.50
Ammonium chloride	0.50
Mineral premix[1]	0.03
Chemical composition, % dry matter	
Dry matter	61.12
Organic matter	95.37
Crude protein	11.25
Neutral detergent fiber	34.85
Acid detergent fiber	15.74
Ether extract	4.67
Total amino acids	7.47
Amino acid composition, % total amino acids	
Glutamic acid	16.61
Leucine	11.45
Alanine	9.01
Proline	9.00
Aspartic acid	7.40
Valine	5.31
Phenylalanine	5.16
Glycine	5.10
Lysine	4.68
Isoleucine	4.19
Arginine	4.18
Serine	4.05
Threonine	4.05
Tyrosine	3.00
Histidine	2.44
Methionine	1.88
Cysteine	1.75
Tryptophan	0.77

[1]Provided to diet (dry matter basis) 11.0 mg/kg Zn, 0.4 mg/kg Co, 44.4 mg/kg ethylenediamine dihydroiodide, 20.3 mg/kg Fe, 38.7 mg/kg Mn, 4.2 mg/kg Na, 5528 IU of vitamin A/kg, 361 IU of vitamin D/kg, and 15 IU of vitamin E/kg.

to visualize effects of rinsing, freezing and lyophilizing on surface structure of lipid-coated lysine.

Experiment 2

Nine abomasally cannulated ewes (70.1 ± 5.2 kg; 5.3 ± 0.6 yr) were used in an experiment to measure lysine bioavailability from feeding EB, EC, and lysine-HCl to ruminants by a slope-ratio analysis (Roach et al., 1967; Finney, 1978; Batterham et al., 1979; Elwakeel et al., 2012). Ewes were individually housed (1.8 m × 0.72 m) in a temperature (18°C) and light (16 h light daily) controlled room, and were limit-fed (1.6-times the calculated maintenance metabolizable energy requirement; NRC, 2007) twice daily (0730 and 1930 h). The diet was designed to meet or slightly exceed requirements for protein (NRC, 2007). Diet composition is reported in Table 1. Samples (100 g) of the diet were collected daily and composited by period and immediately frozen at –20°C.

Ewes were surgically fitted with abomasal catheters as described by Freetly et al. (2010) at least 14 d prior to the experiment to allow abomasal infusion of lysine. Briefly, abomasal catheters were constructed in the laboratory using 1 m of flexible polymer tubing (0.8 cm o.d., 0.5 cm i.d.; Tygon, Saint-Gobain Performance Plastics, Akron, OH) that had a pair of cuffs constructed from slightly larger flexible polymer tubing (1.0 cm o.d., 0.5 cm i.d.) placed 2.54 cm apart to create a 5 cm tip. Subsequently, catheters were surgically placed by dissection of the fundus under general anesthesia using isoflurane, and the tip of catheters were inserted in the abomasum and a purse string suture was placed between each cuff. Catheters were exteriorized 1 cm ventral to the transverse process of the L3 vertebra, and a permanent cuff (1.0 cm o.d., 0.5 cm i.d.) was fixed to the external portion of the catheter to prevent retraction into the abdominal cavity.

Ewes were placed in a 9 × 9 Latin square and treatments included control (no lysine supplementation) as well as 5 or 10 g/d of lysine from EB, EC, or lysine-HCl mixed with the diet 30 min prior to feeding. Additions of EB, EC, or lysine-HCl to the diet was divided evenly between feedings. Abomasal infusion of 5 or 10 g/d lysine acted as a positive control. Abomasal lysine was prepared and delivered daily in 2.6 L of distilled water via a peristaltic pump. When ewes did not receive abomasal lysine infusion 2.6 L/d of distilled water alone was abomasally infused. Each period consisted of 7 d; 6 d for adaptation and measures of plasma amino acids were collected on Day 7 via jugular venipuncture 4 h after the morning feeding. Blood samples were placed on ice after collection, immediately transferred to the laboratory and plasma was harvested by centrifugation (2,200 × *g* at 4°C) for 15 min. Plasma was stored frozen at –20°C until subsequent analysis of lysine via ultra-high performance chromatography. Ewe weights were collected immediately following blood collection and used to calculate the subsequent periods daily feed offering.

Diet samples were thawed at room temperature (18°C), dried (55°C) in a forced-air oven for 48 h and ground to pass a 1 mm screen (Thomas-Wiley laboratory mill model 4; Thomas Scientific USA, Swedesboro, NJ) prior to analyses of dry matter, organic matter, crude protein, neutral detergent fiber, acid detergent fiber, and ether extract. Dry matter content was determined by drying samples at 105°C for 24 h in a forced-air oven. The wet chemistry techniques of Van Soest et al. (1991) were used

to quantify neutral detergent fiber and acid detergent fiber non-sequentially; diet neutral detergent fiber was measured in the presence of sodium sulfite and with addition of α-amylase. Diet samples were analyzed for ether extract (procedure AM 5–04; AOCS, 2005; Ankom XT10; Ankom Technology). Nitrogen content of diet samples were determined by combustion (method 968.06; AOAC International, 2012; Elementar Rapid MAX N exceed; Elementar, Langenselbold, Germany), and crude protein was calculated as 6.25 × N. Amino acid concentration of diet samples was analyzed by high performance liquid chromatography (method number 982.30; AOAC International, 2006). Prior to measures of methionine and cysteine in diet, the sulfur containing amino acid were oxidized with performic acid, and tryptophan was determined from diet samples after alkaline hydrolysis (method number 982.30; AOAC International, 2006).

Plasma lysine content was analyzed for free lysine by reversed phase ultra-high performance liquid chromatography after pre-column derivatization of amino acids with o-phthaldialdehyde (Dai et al., 2014). Prior to chromatography, 2 mL of plasma was mixed with 2 mL of 10% sulfosalicylic acid containing 1 mM norvaline as an internal standard for amino acid analysis. After cooling on ice for 30 min., samples were vortexed and centrifuged (13,800 × g). The supernatant was analyzed and chromatography was achieved on a C_{18} column (3.0 × 150 mm, 3.5 µm; Agilent Corp, Santa Clara, CA) after passing a C_{18} guard column (2.1 × 12.5 mm, 5 µm). The combined flow rate of the mobile phase was constant and 0.64 mL/min. The initial mobile phase (A) was composed of water containing 9.87 µmol/L Na_2HPO_4, 18.89 µmol/L $Na_2B_4O_7$, and 0.49 µmol/L NaN_3. The second mobile phase (B) was composed of water containing 8.58 mol/L acetonitrile and 11.11 mol/L methanol. The percentage of mobile phase A was as follows: 0 min, 98%; 20 min, 43%; 20.1 min, 0%; 23.6 min, 98%. The column was maintained at 40°C and injection volumes were 16 µL. Amounts of plasma lysine were quantified in reference to norvaline and measured at 338 nm (bandwidth = 10 nm) and the reference wavelength was 390 nm (bandwidth = 20 nm) with a diode array detector (Ultimate 3000; Thermo Electron North America, West Palm Beach, FL). Prior to analyses of plasma amino acids, replicate amino acid standards at 15, 30 and 60 µmol/L were analyzed to evaluate precision of analysis. The intra-assay coefficients of variation were 1.7 ± 0.46 for aspartic acid, 1.5 ± 0.41 for glutamic acid, 1.2 ± 0.44 for serine, 1.2 ± 0.11 for histidine, 1.6 ± 0.42 for glycine, 1.3 ± 0.42 for threonine, 1.6 ± 0.42 for arginine, 1.8 ± 0.26 for alanine, 1.7 ± 0.26 for tyrosine, 2.4 ± 0.15 for valine, 2.1 ± 0.04 for methionine, 2.1 ± 0.19 for phenylalanine, 2.0 ± 0.25 for isoleucine and 2.6 ± 0.19 for lysine. Additionally, the area response was linear for all amino acids and the square correlation coefficients (R^2) were 0.9983 for aspartic acid, 0.9977 for glutamic acid, 0.9996 for serine, 0.9991 for histidine, 0.9989 for glycine, 0.9996 for threonine, 0.9993 for arginine, 0.9991 for alanine, 0.9981 for tyrosine, 0.9963 for valine, 0.9964 for methionine, 0.9966 for phenylalanine, 0.9949 for isoleucine and 0.9963 for lysine.

Calculations. Slopes for each treatment were calculated by regressing plasma lysine against amounts of lysine provided in feed or abomasally infused. Subsequently, lysine bioavailability was calculated as the ratio of the slopes of EB, EC or lysine-HCl to that of abomasal lysine infusion (Roach et al., 1967; Finney, 1978; Batterham et al., 1979; Elwakeel et al., 2012).

Statistical Analyses. Plasma lysine was regressed on amounts of lysine added from EB, EC, lysine-HCl, or abomasal infusion using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC); sheep and period were random class variables, and amounts of lysine within source of supplemental lysine were regression variables. Plasma amino acid concentration was analyzed as a Latin square using the MIXED procedure of SAS; the model included effects of treatment and effects of animal and period were considered random. Denominator degrees of freedom were calculated by the Kenward and Roger adjustment (Kenward and Roger, 1997). Effects of added lysine to feed from EB, EC, lysine-HCl or abomasal infusion were determined by linear contrasts.

RESULTS AND DISCUSSION

Experiment 1

Block and Jenkins (1994) observed large amounts of lysine loss from lipid-associated lysine during ruminal incubation. These authors (Block and Jenkins, 1994) speculated that lysine associated with the surface of the lysine-lipid particle was rapidly solubilized during ruminal incubation and that diminished surface integrity of the lysine-lipid particle allowed movement of ruminal fluid throughout the lysine-lipid particle. Reduced integrity of the lipid-lysine particle may also allow movement of water throughout the lysine-lipid particle when mixed with diets that contain large amounts of moisture and increase lysine lost from lipid-coated lysine. Ji et al. (2016) reported that increased exposure to a silage-based diet increased amount of lysine loss from lipid-coated lysine products. Further, we (Reiners and Brake, 2016) previously reported that amounts of lysine lost from lipid-associated lysine affected by silage type, acidity, and manufacture; however, we are not aware of any effort to image lipid-associated lysine surface structure after mixing lipid-associated lysine with alfalfa or corn silage containing different amounts of acidity.

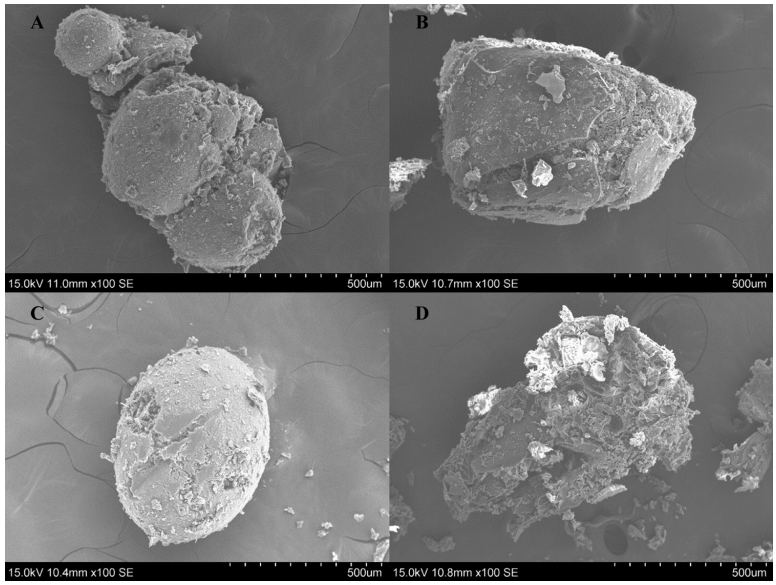

Figure 1. Scanning electron microscropy of lipid-coated lysine after 24 h incubation in alfalfa silage. Panel A: Scanning electron micrograph of EB (lipid-coated lysine that contained 47.5% lysine-HCl and 52.5% lipid) after incubation in low pH alfalfa silage. Panel B: Scanning electron micrograph of EC (lipid-coated lysine that contained 65% lysine-HCl and 35% lipid) incubated in low pH alfalfa silage. Panel C: Scanning electron micrograph of EB incubated in neutral pH alfalfa silage. Panel D: Scanning electron micrograph of EC incubated in neutral pH alfalfa silage.

Surface structures of lipid-coated lysine appeared to be impacted by mixing it with alfalfa- (Fig. 1) or corn silage (Fig. 2) in comparison coated lysine that was not mixed with silage (Fig. 3). Evaluation of scanning electron micrographs of lipid-coated lysine not exposed to silage, rinsing, freezing and lyophilizing compared to lipid-coated lysine that was rinsed, frozen and lyophilized but not exposed to silage indicate that rinsing, freezing and lyophilizing had little impact on lipid-coated lysine surface structures. Additionally, scanning electron micrographs seemed to indicate that the surface structure of lipid-associated lysine mixed with alfalfa silage was degraded equally despite differences in silage pH; however, the surface structure of lipid-associated lysine mixed with corn silage appeared to be more disrupted when mixed with more acidic corn silage in comparison to less acidic corn silage. Indeed, these surface structure images appear to be in agreement with our previous observation (Reiners and Brake, 2016) that greater acidity increased amounts of lysine lost from lipid-associated lysine mixed

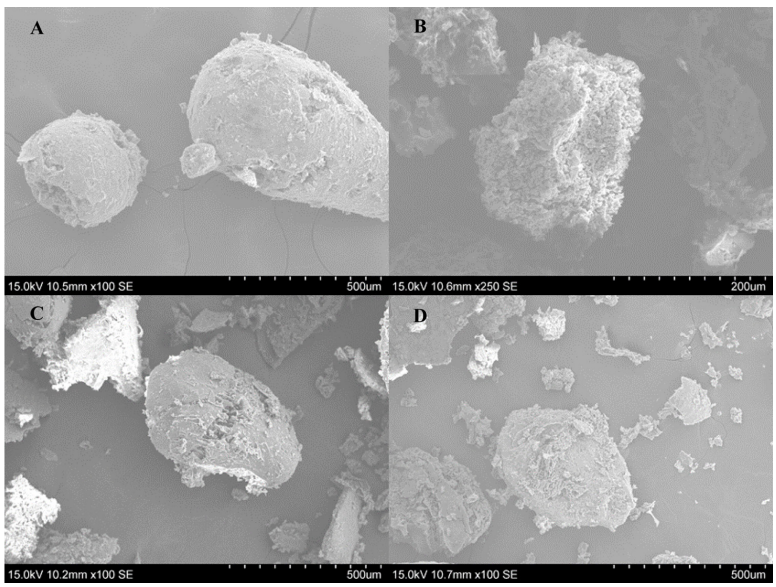

Figure 2. Scanning electron microscopy of lipid-coated lysine after 24 h incubation in corn silage. Panel A: Scanning electron micrograph of EB (lipid-coated lysine that contained 47.5% lysine-HCl and 52.5% lipid) incubated in low pH corn silage. Panel B: Scanning electron micrograph of EC (lipid-coated lysine that contained 65% lysine-HCl and 35% lipid) incubated in low pH corn silage. Panel C: Scanning electron micrograph of EB incubated in neutral pH corn silage. Panel D: Scanning electron micrograph of EC incubated in neutral pH corn silage

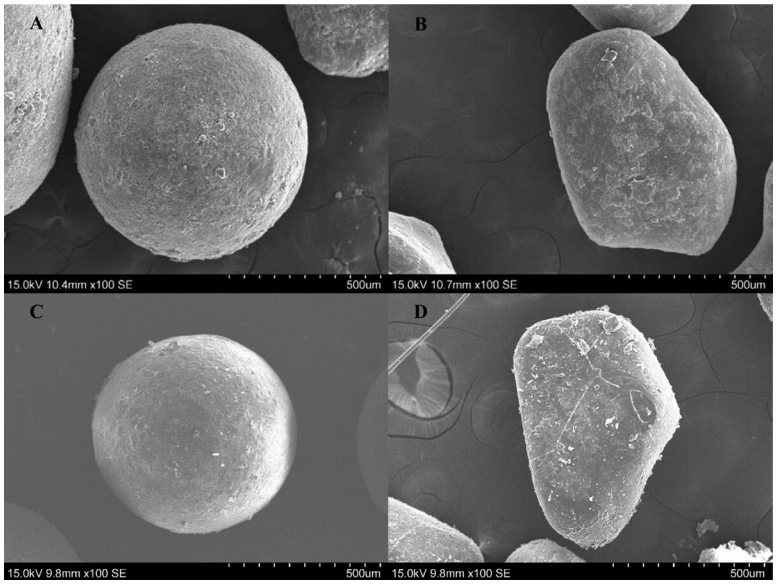

Figure 3. Scanning electron microscopy of lipid-coated lysine products prior to incubation in silages. Panel A: Scanning electron micrograph of EB (lipid-coated lysine that contained 47.5% lysine-HCl and 52.5% lipid). Panel B: Scanning electron micrograph of EC (lipid-coated lysine that contained 65% lysine-HCl and 35% lipid). Panel C: Scanning electron micrograph of EB not exposed to silage but after rinsing with water, freezing and lyophilizing. Panel D: Scanning electron micrograph of EC not exposed to silage but after rinsing with water, freezing and lyophilizing.

with corn silage but that acidity had little impact on amount of lysine lost from lipid-associated lysine mixed with alfalfa silage. Obviously, surface structure images are subjective measures, but these images appear to agree with the report of Ji et al. (2016), our previous observations (Reiners and Brake, 2016) and the conclusions of Block and Jenkins (1994). Regardless, images from this preliminary study clearly indicate that measures of lysine bioavailability are necessary to allow an improved understanding of amounts of metabolizable lysine provided from lipid-coated lysine after exposure to silage.

Experiment 2

Rulquin and Kowalczyk (2003) reported that abomasal infusion of graded amounts of lysine to lactating cows and subsequent measurement of plasma lysine is more effective than in vitro procedures to determine amounts of metabolizable lysine from lipid-coated lysine. We used 9 mature ewes fitted with abomasal catheters and fed diets designed to not be limiting in lysine to evaluate amounts of metabolizable lysine provided from lipid-coated lysine or lysine-HCl after mixing with corn silage.

Plasma lysine concentrations (Fig. 4) increased linearly ($P < 0.01$) in response to abomasal infusion of lysine indicating that our model was sensitive to increases in metabolizable lysine flow. Bioavailability of EC, EB, and lysine-HCl in feed was calculated as the ratio of the rate of plasma lysine increase from EC, EB, or lysine-HCl in feed to the rate of plasma lysine increase in response to known amounts of lysine infused to the abomasum. Our data indicate that bioavailability of lysine from EC, EB, and lysine-HCl in feed was 23, 15, and 18%, respectively. Even though each source of lysine provided a positive rate of increase among plasma lysine sources, rates of increase in plasma lysine from EB (linear; $P = 0.20$) and lysine-HCl (linear; $P = 0.11$) were not different from plasma lysine levels supported by diet alone. However, the rate of plasma lysine increase in response to lysine from EC was greater (linear; $P = 0.04$) than plasma lysine from feed alone. Yet, the rate of plasma lysine increase in response to lipid-coated lysine did not differ ($P \geq 0.70$) from the rate of plasma lysine increase from lysine-HCl.

Effects of feeding lipid-coated lysine on plasma lysine concentration and lactation performance among lactating cows fed silage-based diets are variable. Several authors (Robinson et al., 2011; Giallongo et al., 2016) reported increases in plasma lysine and milk protein content when lactating cows were supplemented with lipid-coated lysine. Conversely, others (Swanepoel et al., 2010; Robinson et al., 2010; Lee et al., 2012, 2015) reported no effect of lipid-coated lysine on plasma lysine and milk protein concentration. Authors have speculated (Robinson et al., 2010; Swanepoel et al., 2010) that a lack of response among plasma lysine or milk protein may have been related to changes in partitioning of amino acids for physiological functions other than milk protein production or because diets were not first-limited by metabolizable lysine. However, Wu et al. (2012) calculated that only 11.5% of lysine from lipid-coated lysine was digested in the small intestine of cows when they measured lysine loss from lipid-coated lysine in mobile

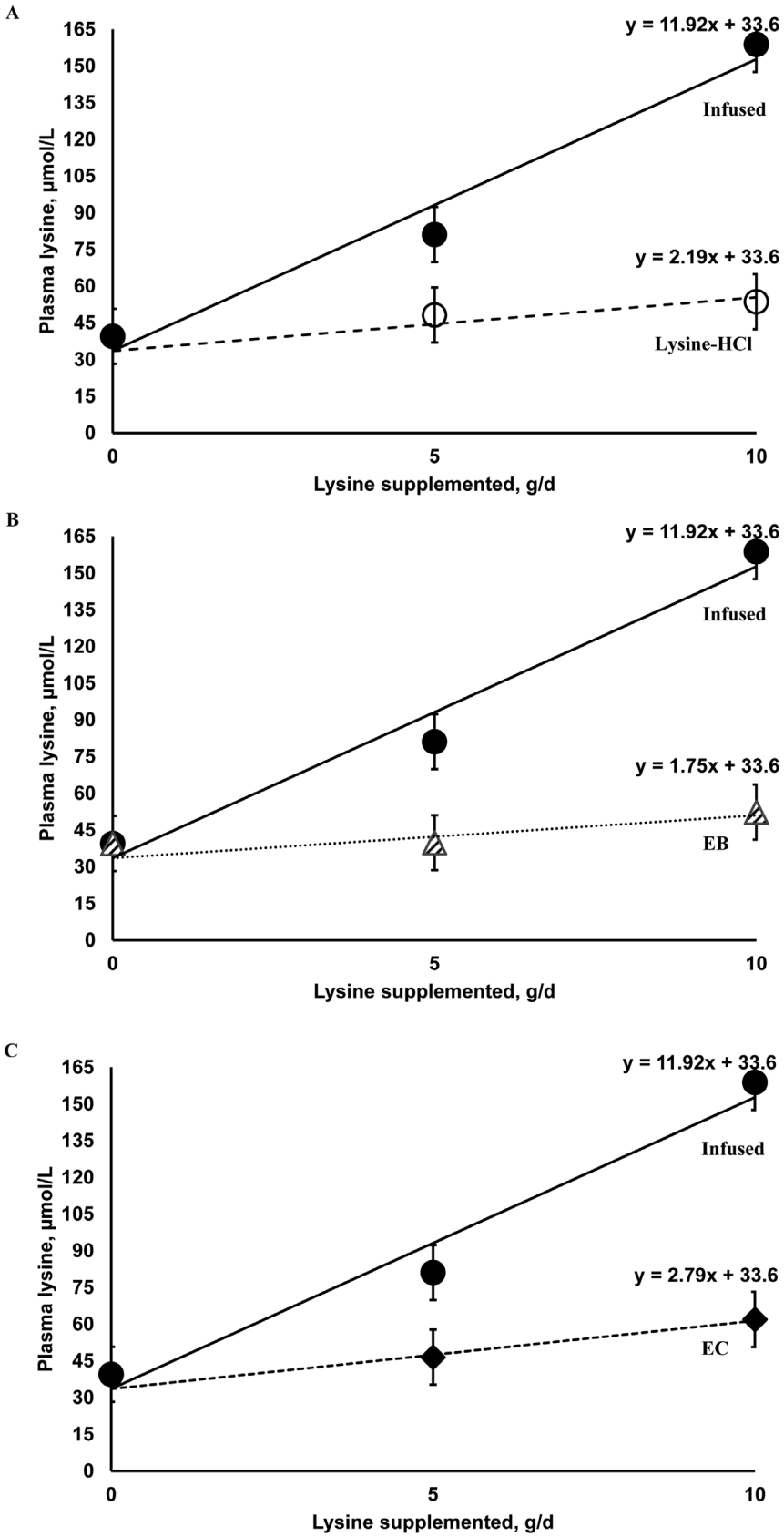

Figure 4. Plasma lysine concentration in ewes provided lysine as an abomasal infusion of lysine-HCL or as dietary lysine-HCl (Panel A), EB (Panel B; lipid-coated lysine that contained 47.5% lysine-HCl and 52.5% lipid), or EC (Panel C; lipid-coated lysine that contained 65% lysine-HCl and 35% lipid). Standard error of estimate = 1.3.

Table 2. Effect of 0, 5 or 10 g/d lysine from abomasal infusion or from lysine-HCl and 2 lipid-coated lysine products added to a corn silage-based diet on plasma amino acid concentrations (μmol/L) in sheep

	Control	Infused		Lysine-HCl		EB[2]		EC[3]			Linear contrasts			
AA[1]	None	5	10	5	10	5	10	5	10	SEM	Infused	Lysine-HCl	EB	EC
Glu	28.8	25.3	26.0	29.5	27.7	22.3	24.1	30.6	23.5	3.9	0.35	0.71	0.11	0.07
Asp	20.5	16.0	17.0	19.4	17.8	18.3	19.9	17.9	17.1	1.8	0.06	0.14	0.75	0.07
Ser	21.1	18.3	21.0	19.9	18.4	18.8	20.6	23.0	17.6	1.5	0.94	0.12	0.78	0.04
Gln	252.3	232.8	250.0	257.7	226.7	228.6	264.0	263.0	232.7	16.2	0.88	0.11	0.46	0.22
His	49.4	46.6	45.2	47.7	45.6	47.6	48.0	50.1	48.3	3.7	0.23	0.27	0.68	0.75
Gly	123.9	109.1	110.3	110.6	109.5	114.0	128.2	119.9	107.6	9.0	0.09	0.07	0.58	0.04
Thr	32.8	26.0	25.3	25.4	31.8	28.4	28.3	30.9	25.1	3.9	0.08	0.82	0.29	0.08
Arg	66.6	66.4	78.5	64.9	61.0	63.0	68.3	64.9	64.9	5.8	0.06	0.38	0.80	0.79
Ala	45.9	44.9	47.2	46.0	40.8	42.4	43.6	45.9	41.0	3.1	0.72	0.14	0.51	0.15
Tyr	26.1	21.3	20.3	24.6	24.8	24.5	23.0	24.4	20.9	2.1	<0.01	0.47	0.10	0.01
Val	40.3	42.9	43.3	42.3	40.9	41.6	42.8	41.8	38.6	4.7	0.52	0.90	0.59	0.72
Phe	17.9	16.3	14.6	17.4	16.9	17.0	15.9	16.5	15.5	1.5	0.01	0.42	0.12	0.07
Ile	22.3	21.3	21.8	21.7	19.8	20.4	21.0	19.6	19.4	2.5	0.82	0.30	0.60	0.23
Leu	28.3	27.3	27.1	27.5	24.9	26.7	28.0	26.9	21.3	3.0	0.67	0.19	0.91	0.01
Lys	39.5	81.1	158.8	48.2	53.7	39.9	52.3	46.5	62.0	11.3	<0.01	0.36	0.41	0.15

[1]Amino acids are described by standard 3 letter abbreviations.

[2]Lipid coated lysine that contained 47.5% lysine-HCl and 52.5% lipid.

[3]Lipid coated lysine that contained 65% lysine-HCl and 35% lipid.

bags, and Paz and Kononoff (2014) speculated that a lack of response in arterial lysine concentration among cows fed lipid-coated lysine was because lipid-coated lysine provided less metabolizable lysine than expected. Regardless of the reason for a lack of response to lipid-coated lysine, it cannot be discounted that one explanation to a lack of performance response to lipid-coated lysine may be that amounts of metabolizable lysine from lipid-coated lysine were less than anticipated.

Rulquin and Kowalczyk (2003) concluded that use of lipid-coated lysine may be useful to evaluate bioavailability of amino acids from feed. However, our data together with others (Ji et al., 2016) emphasize caution when using lipid-coated lysine as a positive control in determining estimates of lysine bioavailability, and that lipid-coated lysine should only be used as a positive control after effects of physical and chemical characteristics of diet on availability of lysine from lipid-coated lysine has been validated.

Generally, slope ratio analyses represent direct measures of an amino acid's availability to non-splanchnic tissues (McNab, 1994; Moehn et al., 2005). Thus, it is surprising that only a limited number of reports are available that have measured amino acid availability from "ruminally-protected" amino acid products to ruminants. Our data indicate that relatively modest amounts of metabolizable lysine were available to sheep from lipid-coated lysine mixed with corn silage. It is possible that measures of metabolizable lysine from lipid-coated lysine could differ between ruminant species; however, our measures of lysine bioavailability are similar to amounts of lysine apparently available to be digested in the small intestine of cows (Wu et al., 2012).

Plasma amino acid concentrations are reported in Table 2. The only amino acids affected by treatment other than lysine were modest changes in serine, tyrosine, phenylalanine, leucine, and glycine. Specifically, increased intake of EC and greater abomasal infusion of lysine reduced (linear; $P \leq 0.01$) plasma tyrosine. Greater amounts of lysine from EC decreased (linear; $P = 0.03$) plasma serine, leucine, and glycine. Additionally abomasal infusion of lysine decreased (linear; $P = 0.01$) plasma phenylalanine. A specific explanation for slight changes in circulating concentrations of serine, tyrosine, phenylalanine, leucine, and glycine remains unknown. Evidently, increases in both metabolizable lysine and ruminally degraded lysine can impact circulating amino acid concentrations.

Conclusions

Our data indicate that lipid-coated lysine can provide metabolizable lysine to ruminants, but that amounts of metabolizable lysine from lipid-coated lysine are affected by both physical and chemical characteristics of diet. More direct measurements of lysine availability to ruminants are needed to improve estimates of metabolizable lysine from lipid-coated lysine products in various feeding conditions, and it is possible that variation in production responses to lipid-coated lysine are related to inaccurate estimates of amounts of metabolizable lysine provided from lipid-coated lysine. Reduced exposure of lipid-coated lysine

to silage-based or acidic diets may augment amounts of metabolizable lysine provided to ruminants.

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