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Short communication: Lysine retained among 2 lipid-coated lysine products after exposure to alfalfa or corn silage with different amounts of acidity

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ABSTRACT

We conducted 2 experiments to determine lysine loss from 2 lipid-coated lysine products after mixing with silage. In our first experiment, we mixed 2 lipid-coated lysine products, crystalline lysine or crystalline lysine and amounts of lipid identical to amounts included in lipid-coated lysine products, with alfalfa or corn silage that had 2 different amounts of acidity. Lysine appeared to disassociate from lipid-coated lysine products in a nonlinear manner after mixing with either alfalfa or corn silage at different amounts of acidity. Additionally, silage source and acidity affected amounts of lysine released from lipid-coated lysine products after mixing. In a corresponding experiment, in vitro estimates of lysine available to ruminal microbiota after mixing with alfalfa or corn silage at different amounts of acidity were measured by ammonia release. In vitro measures were conducted with or without monensin to allow estimates of effects of monensin on amounts of lysine released from the 2 lipid-coated lysine products. It is unclear whether in vitro estimates of lysine fermentation from lipid-coated lysine are truly reflective of ruminal degradation of lysine from lipid-coated lysine because amounts of time needed to measure differences between different lysine sources were greater than typical estimates of mean ruminal particulate retention time. Nonetheless, monensin apparently reduced ammonia release from lysine, but ammonia release from lipid-coated lysine did not differ from crystalline lysine. Clearly, methods of manufacture together with physical and chemical characteristics of diet can affect amounts of lysine provided from lipid-coated lysine products to ruminants.

Key words: acidity, cattle, ruminally protected lysine, silage

Short Communication

To be an effective source of metabolizable AA, ruminally protected AA products must withstand mixing, innate chemical characteristics of the diet, mastication, and ruminal fermentation (Papas and Wu, 1997). Ji et al. (2016) reported that mechanical mixing, diet moisture content, and increased amounts of time exposed to feed decreased lysine retained in several lipid-coated lysine products (**LCLys**). Hydrolysis of triglycerides to fatty acids and glycerol can be catalyzed by acid (Bender et al., 1961; Carey, 2003), and acid-catalyzed hydrolysis is greater in environments with large water concentrations (Le Chatelier, 1884; Bender et al., 1961; Carey, 2003). Thus, it is possible that diets containing large amounts of silage and, concomitantly, large amounts of water and organic acids may affect amounts of AA retained by LCLys. Our objective in this work was to determine effects of acidity and silage source on lysine retained by 2 LCLys products and to estimate ruminal degradation of lysine retained by LCLys after mixing with alfalfa or corn silage with different amounts of acidity. All procedures involving the use of animals were approved by the South Dakota State University Institutional Animal Care and Use Committee.

In an initial experiment we evaluated amounts of lysine retained by 2 LCLys products after exposure to alfalfa or corn silage with different pH levels. Prior to in situ incubation of LCLys in silage, alfalfa (50% DM; 741 kg) was collected, wilted, and chopped (average particle size = 0.81 cm) with a hammer mill (Patriot Pro-Series model CSV-3090H; Patriot Products Inc., Pewaukee, WI) before it was packed (0.7 ± 0.05 kg/L) into 2 miniature silos (121 L) and ensiled for 169 d. Similarly, freshly chopped (average particle size = 0.79 cm) corn plants (46% DM; 334 kg) were collected and packed (0.6 ± 0.05 kg/L) into 2 miniature silos (121 L) and ensiled for 154 d.

After ensiling, corn silage (pH = 3.7 ± 0.1) and alfalfa silage (pH = 4.4 ± 0.1) from each of the 2 silos were composited by silage type, and pH was measured immediately before in situ incubation of LCLys in silage. The pH of each composited silage was measured

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as described by Buchanan-Smith and Yao (1981). After initial pH measurement, silage pH was adjusted by mixing (model 2030, Marion Mixer, Marion, IA) 10% (wt/wt) NaOH for 5 min to achieve the appropriate acidity. Acidity in an aliquot of corn silage (11 kg of DM) was modified to be similar to the initial pH of alfalfa silage (pH = 4.6) by addition of 1.55 kg of NaOH. Subsequently, an aliquot of alfalfa silage (9.5 kg of DM) was mixed with 2.80 kg of NaOH to increase pH to 6.8. Additionally, another aliquot of corn silage (11 kg of DM) was mixed with 3.15 kg of NaOH to achieve a similar pH (pH = 6.9) to alfalfa silage with added NaOH. Modification of silage pH allowed for evaluation of effects of acidity on lysine loss from LCLys by creating acidic corn silage (pH = 4.6) and alfalfa silage (pH = 4.4) and more neutral corn silage (pH = 6.9) and alfalfa silage (pH = 6.8).

Samples (4 g) of 2 LCLys products were placed in duplicate polyester bags (10 × 20 cm, pore size = 50 μm; Dacron, Ankom Technology, Fairport, NY) and heat sealed before placement in silage. One LCLys product (**LP**; LysiPearl, Kemin Industries, Des Moines, IA) consisted of 47.5% lysine-HCl and 52.5% lipid and was manufactured by embedding lysine-HCl into a lipid matrix. The second LCLys (**USA**; USA Lysine, Kemin Industries) consisted of 65% lysine-HCl and was manufactured by extrusion of lysine-HCl and lipid into small particles and subsequently coating lysine lipid particles with multiple layers of a lipid matrix. Lysine-HCl (4 g) alone served as a negative control. Additionally, lysine-HCl and the same lipid used to manufacture LP and USA (58.1% palmitic acid, 39.2% stearic acid, 1.3% myristic acid, 0.5% arachidic acid, 0.3% lauric acid, 0.2% heptadecanoic acid, 0.1% lignoceric acid, 0.1% linoleic acid, 0.1% pentadecylic acid) were added separately to the same polyester bags in amounts identical to either LP or USA and served as controls to evaluate manufacturing method. After silage acidity was adjusted, replicate polyester bags were hand mixed with corn silage (22.7 kg) or alfalfa silage (11.4 kg) in a plastic container (191 L; length = 108.6 cm; width = 55.9 cm; height = 45.7 cm) and immediately removed (i.e., 0 h) or incubated for 6, 12, or 24 h in a temperature-controlled room (20°C). At the appropriate time, bags were removed and rinsed with 5 L of cold tap water per side over a 40-μm screen. After rinsing, samples were frozen (−20°C) and lyophilized before removal from polyester bags.

Lipid-associated lysine content was analyzed from lyophilized samples as described by Brake et al. (2013). Linear and nonlinear models were used to predict rates of lysine disassociation from LCLys after mixing with silage. Linear estimates of lysine disassociation were calculated as the slope of the regression line of the natural logarithm of lipid-associated lysine in LCLys versus incubation time (Mathers and Miller, 1981; Bach et al., 1998). Additionally, we evaluated a first-order kinetic model to predict rates of lysine disassociation (Ørskov and McDonald, 1979). The first-order kinetic model calculated lysine disassociation as the sum of the proportion of lysine that immediately disassociates from LCLys (**ID**) and the product of the proportion of lysine that is potentially dissociable (slowly disassociates; **SD**). The amount of lysine disassociation from SD was calculated as 1 minus the logarithm of the product of the hourly rate of disassociation ($\mathbf{K_d}$) and time. The equation was fitted using the Marquardt method for iterative, nonlinear, least squares estimation in SAS (SAS Institute Inc., Cary, NC). Incorporating lag time into the model did not seem appropriate from the shape of the degradation curves (Figure 1). Because a coefficient of determination is not readily defined in nonlinear regression, we calculated pseudocoefficients of determination as 1 − (sum of squared residuals/corrected total sum of squares). Extent of lysine disassociation was calculated as the sum of ID and the product of SD and the quotient of K_d and K_d plus the rate of diet intake (Ørskov and McDonald, 1979). Because rate of diet intake was assumed to be constant across time (Hart et al., 2014; Yuan et al., 2015), rate of diet intake was calculated as the reciprocal of 8, 12, 16, and 24 h, respectively. Subsequently, amounts of lysine that remained associated with each LCLys product were calculated as 1 − extent of lysine disassociation.

Both the linear and nonlinear models were tested for goodness of fit by the lack-of-fit test described by Sokal and Rohlf (1969). Appropriateness of the linear model was evaluated by plotting residuals versus fitted values, and coefficients of determination were calculated with the REG procedure of SAS. Pool sizes (ID, SD, and lysine not disassociated) and K_d were analyzed as a completely randomized design using the MIXED procedure of SAS. The model contained effects of LCLys, silage type, pH, and all interactions.

We conducted an additional experiment to estimate ruminal degradation of lipid-associated lysine in each LCLys product with or without monensin. Samples (4 g) of LP and USA were placed in polyester bags (10 × 20 cm; pore size = 50 μm) and hand mixed with an aliquot of the same alfalfa silage (pH = 4.4; 11.4 kg) and corn silage (pH = 3.7; 22.7 kg) used in Experiment 1 before addition of NaOH. Polyester bags containing LP and USA were removed after 30 min, rinsed with 5 L of cold tap water per side over a 40-μm screen, frozen (−20°C), and subsequently lyophilized inside polyester bags.