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Technical Literature

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Determining bioavailability of LysiGEM™ Extend prototypes using the *in vivo* plasma dose response method[1]

Abstract

LysiGEM™ Extend is a rumen-protected lysine product developed by Kemin Animal Nutrition and Health. In this study, the plasma dose response method was used to determine the bioavailability in eight lactating multiparous Holsteins who were fed LysiGEM™ Extend prototypes. The experiment was done using a 4 × 4 Latin square study with 7-day treatment periods. The four treatments used included positive and negative controls as well as two prototypes of LysiGEM™ Extend, A and B. LysiGEM™ Extend prototypes were mixed with 1.5 kg of Total Mixed Ration (TMR) and placed in tubs in front of the cows 30 minutes before each of the 3 daily feedings. Blood samples were obtained from each cow on the last 3 days of each covariate and experimental periods, then analyzed for lysine and other amino acid (AA) concentration. The bioavailability of LysiGEM™ Extend prototypes was calculated by comparing the slopes of the infused and fed LysiGEM™ Extend Prototypes (Lys as % of total AA). The bioavailability was 35.5% and 49.0% for Prototypes A and B, respectively. Prototype B exhibited higher bioavailability. This data was used to determine the final LysiGEM™ Extend prototype.

KEYWORDS

LysiGEM™ Extend; plasma dose response; bioavailability study

Introduction

Lysine is an essential amino acid added to the diets of dairy cows to increase milk protein yield. To further raise this yield, lysine is typically delivered using a rumen protected supplement.[2,3] Kemin Animal Nutrition and Health developed LysiGEM™ Extend- a rumen-protected lysine product, which utilizes a granular lysine hydrochloride core coated with a protective layer. To test the product's efficacy in dairy diets, a bioavailability trial was conducted using the plasma-dose response technique. This technique, also called the slope-ratio assay, is a replicated 4 x 4 Latin square design consisting of 7-day periods where cows receive one diet every 7-days for a total of 4 weeks.[4] Two LysiGEM™ Extend prototypes were studied using this assay: Prototype A contained 63.76% lysine and the Prototype B contained 63.63% lysine. These protypes were placed in total mixed rations (TMR) for 30 minutes before being fed to the cows. Lysine HCl (Lys-HCl, 99%), which acted as a positive control, was delivered directly into the abomasum via an infusion line, except during the milking periods. This is a common practice in this assay to make sure that the cows nutritional needs are being met and their energy is not a limiting factor.

The plasma-dose technique was conducted by the University of New Hampshire (UNH) using eight multiparous lactating Holsteins (128 ± 38 days in milk (DIM)) during the summer of 2022. In addition to calculating the bioavailability of the two LysiGEM™ Extend prototypes, a series of other tests were investigated to understand the nature of these rumen protected lysine products including chemical compositions of feed samples, blood and milk samples.

Materials and Methods

Experimental Design

Experimental treatments are shown in Table 1 below. Prototypes were fed to achieve 60 g/d of supplemental lysine, which is commonly used to satisfy >25 kg of daily milk production.[5] Prototype A and Prototype B were produced via Kemin's proprietary processing and manufacturing procedures. Both prototypes had the same lysine core which consisted of lysine hydrochloride, but differed in the coating formulation.

Table 1: Experimental treatments used in the study

Treatment	Amount fed (g/d)	Description
1	0	Negative Control
2	60	Positive Control - abomasally infused Lys-HCl
3	60	Prototype A
4	60	Prototype B

For the infusion treatment, lysine- hydrochloride (Lys-HCl) was mixed with 4 L of hot tap water until completely dissolved. The lysine solutions were continuously infused (175 mL/h) into the abomasum via the rumen cannula using a peristaltic pump (Masterflex, Cole-Parmer, Vernon Hills, IL). The pump was turned off and the infusion lines disconnected only when cows were moved from their stalls to the milking parlor. Fresh infusion solutions were prepared daily at 1300 h. Pumping rates were adjusted and monitored to ensure uniform and complete daily infusions. The Lys-HCl used for infusions was the same Lys-HCl used in the production of rumen protected- lysine (RP-Lys) supplements.

The rumen-protected amino acid (RP-AA) supplements were mixed with 1.5 kg of total mixed ration (TMR) right before each feeding. To ensure complete consumption of rumen protected-methionine (RP-Met) supplements, and to match its feeding with the TMR feedings, the TMR/RP-AA mix was placed in rubber tubs and fed to the cows 30 minutes before each feeding. Any TMR/RP-AA mix not consumed by the cow by the end of 15-20 minutes was placed in the rumen via the ruminal cannula at similar rates as the infused treatment to ensure complete daily dosing of lysine. Cows were fed 3 times daily (0500, 1300, and 2100h) with 1/3 of their daily feed allotment fed at each feeding. This was done to match the feedings of RP-Met products with the TMR feedings and to maintain a constant ratio of RP-Met products to total TMR consumed.

Cow Management

All procedures related to animal care were conducted under approval of the UNH Institutional Animal Care and Use Committee (190901). Cows were housed in a naturally ventilated tie-stall barn and fed individually. Cows had continuous and free access to water and were milked twice daily (0430 and 1530h).

All cows were fed a Lys-adequate and Met-adequate basal diet throughout the study (Table 2). The basal diet was fed as a TMR and prepared 3 times (0500, 1300, and 2100h) by weighing each ingredient and mixing in a mobile paddle mixer. Cows were fed for ad libitum feed intake with minimal orts (2 to 4%).

Table 2: Ingredient composition of basal Lysine adequate diet

Ingredient	% DM
Corn silage, mature	24.05
Mixed mostly grass silage, mid-maturity	21.23
Steam flaked corn	4.21
Corn meal	15.97
Beet pulp	9.97
Molasses, sugar cane	1.16

Distillers grains with solubles	0.73
Soybean meal, solvent extracted	6.46
Canola meal, solvent	2.16
SoyPlus®	7.54
Urea	0.12
BergaFat®-100	3.06
KESSENT™ Me	0.09
Mineral/vitamin mix	3.24

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BergaFat® is a registered trademark of Berg & Schmidt GmbH (Hamburg, Germany).
KESSENT™ Me is a trademark of Kemin Animal Nutrition and Health (Des Moines, Iowa).

Blood Sampling Analysis

Blood samples were obtained from each cow on the last 3 days of the covariate period and the last 3 days of the experimental periods. Additionally, four blood samples were collected each day from the tail or jugular vein at 2-h intervals starting at 0700h. Blood was collected in 10-mL vacutainer tubes (Monoject, Mansfield, MA) containing 15% K_3EDTA. Tubes were placed immediately in a Chameleon Cooler (Chameleon Cooler Corp, Valparaiso, IN), centrifuged within 15 minutes at 1,200 × g for 20 minutes at 5 °C. A 4-mL aliquot from each sample was placed in a labeled glass test tube containing 1.0 mL of 15% 5-sulfosalicylic acid (SSA). The tubes were allowed to sit for 10 minutes in the cold centrifuge before spinning at 1,200 × g for 20 minutes at 5°C. A 0.45 mL aliquot of deproteinized plasma was removed and placed into 1.8-mL cryovials and stored at -80°C for plasma AA analysis (Experimental Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO) using cation-exchange chromatography. Samples from each day and each cow were combined into one for these analyses.

Statistical Methods

Covariate data was used in the statistical analysis (SAS® 9.2, 2010)(SAS Institute, Inc., Cary, NC). The data was also checked to make sure that there was no carryover of treatment when cows went from the infusion of RP-Lys treatment to control. Using the slope ratio assay,[6] the least square means generated from the MIXED procedure were subjected to the PROC REG procedure to generate the linear regression variables and r.[2] Significant level effects were noted at $P \leq 0.05$ and trends were noted $P > 0.05$ to $P < 0.10$.

Results

Blood and Plasma Analysis

Plasma concentrations of amino acids and selected plasma metabolites are reported in Table 3. There were significant differences in treatments for histidine (His, $P = 0.04$), lysine (Lys, $P < 0.0001$) and threonine (Thr, $P = 0.005$) with the infusion treatment being significantly lower for histidine and threonine compared to the other three treatments. For lysine, the infusion treatment was significantly higher than the other three treatments. There was no difference between treatments with Prototypes A and B, but both were significantly higher than control. There is a trend ($P = 0.09$) for arginine (Arg), ($P = 0.08$) for leucine (Leu) and valine (Val).

Table 3. Selected plasma amino acids (µM) for Holstein cows fed a diet supplemented with RP-Lys supplements

Item	Control	Infusion	Prototype A	Prototype B	SEM	*P*-value
Arginine	85.9	99.4	98.7	96.9	4.71	0.09
Histidine	55.5[a]	51.5[b]	57.2[a]	58.4[a]	1.85	0.04
Isoleucine	127.3	133.8	139.6	135.1	4.35	0.18
Leucine	161.5	166.1	178.1	170.8	5.62	0.08
Lysine	89.6[c]	132.2[a]	109.2[b]	110.3[b]	5.16	<0.0001
Methionine	38.4	36.4	41.1	39.0	1.49	0.19
Phenylalanine	47.0	46.6	49.8	47.8	1.38	0.26
Threonine	126.7[a]	117.9[b]	132.0[a]	129.8[a]	4.94	0.005
Tryptophan	45.0	45.6	47.4	45.7	1.30	0.31
Valine	257.8	262.1	278.6	273.7	7.94	0.08
Alanine	315.3	304.6	307.2	312.8	9.97	0.51

a-c Within a row, least squares means without a common superscript differ (P < 0.05)

Lysine Bioavailability

Lysine as a percentage of total AA minus Lys was used to calculate the bioavailability of Lys shown in Table 4. The bioavailability for Prototype A is 35.5 ± 1.7 and 49.0 ± 3.0 for Prototype B. Figure 1 provides equations which can be used to determine how much lysine is provided by a product at a given level of plasma Lys as a % of (TAA-Lys). Table 5 indicates what the metabolizable Lys of each prototype is with Prototype A providing 226.3 g/kg and Prototype B providing 310.5 g/kg.

Table 4. Bioavailability calculation using changes in plasma Lys % of (TAA-Lys) using commercial values

Item	Infusion	Prototype A	Prototype B
Slope	0.02846	0.01010	0.01394
Bioavailability of RP-Lys[1]	-	35.5 ± 1.7	49.0 ± 3.0

[1]Calculated as [(slope of RP-Lys /slope Infusion) *100]

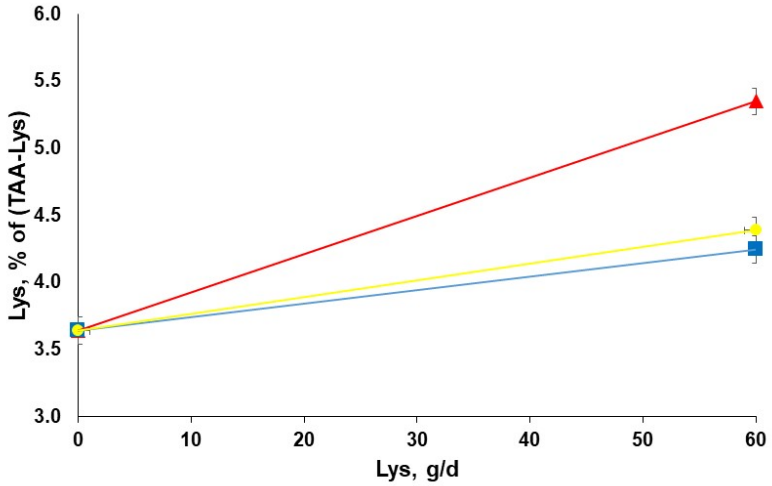

Figure 1. The relationship between Infusion (▲), Prototype A (■), and Prototype B (●) in lactating dairy cows using commercial Lys content. Infusion: Y = 3.63 + 0.0285x; slope SE = 0.001, intercept SE = 0.03, r^2 = 0.99. Prototype A = 3.63 + 0.0101x; slope SE = 0.001, intercept SE = 0.02, r^2 = 0.99; relative bioavailability = (0.0101 ÷ 0.0285) × 100 = 35.5. Prototype B = 3.63 + 0.0139x; slope SE = 0.001, intercept SE = 0.03, r^2 = 0.98; relative bioavailability = (0.0139 ÷ 0.0285) × 100 = 49.0.

Table 5. Metabolizable lysine for the RP-Lys supplements

	Lys, %	Bioavailability, %	Metabolizable Lys, g/kg
Prototype A	63.76	35.5	226.3
Prototype B	63.36	49.0	310.5

Conclusion

The bioavailability of LysiGEM™ Extend Prototypes was successfully determined using the plasma dose response method. The bioavailability was 35.5 ± 1.7 for Prototype A and 49.0 ± 3.0 for Prototype B. Metabolizable Lys was also higher for Prototype B at 310.5 g/kg vs Prototype A at 226.3 g/kg. The bioavailability of LysiGEM™ Extend Prototype B was higher than LysiGEM™ Extend Prototype A.

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