balchem

AminoShure®-XL
Precision Release Lysine

Evaluating Rumen Protected Lysine Products

Multi-Study Research Summary

Trial 1: Bioavailability of multiple rumen-protected amino acids by a stable-isotope technique in dairy cattle.

Fernandes, T., M. H. De Oliveira, A. Hruby-Weston, M. Morozyuk, B. Thelen, and M. D. Hanigan. 2024. J. Dairy Sci. 107(Suppl. 1): 115. (Abstr.)

Trial 2: Evaluating the TMR stability of rumen protected lysine products.

Estes, K., M. Zenobi, C. Zimmerman, and M. Hanigan. 2024. J. Dairy Sci. 107(Suppl. 1): 387. (Abstr.)

Trial 3: Impact of three rumen-protected lysine prototypes on dairy cow performance, milk composition, and milk casein.

Barnard, A.M., B.A. Barton, C.A. Zimmerman, R.S. Ordway, and T.F. Gressley. 2016. J. Dairy Sci. 99(Suppl. 1): 764. (Abstr.)

Trial 4: Assessing bioavailability of ruminally protected methionine and lysine prototypes.

Fleming, A.J., K. A. Estes, H. Choi, B. A. Barton, C. A. Zimmerman, and M. D. Hanigan. 2019. J. Dairy Sci. 102: 4014-4024.

Trial 5: Rumen-protected lysine supplementation increased milk production in dairy cows fed a lysine-deficient diet.

Bailey, H.R., J.D. Kaufman, K.A. Estes, C.A. Zimmerman, B.A. Barton, and A.G. Rius. 2019. Appl Anim Sci. 35(5): 482-490.

Introduction

Lysine is considered one of the first two limiting amino acids (AA) in lactating dairy cows. Ruminally protected lysine products (RP-Lys) are supplemented in rations to increase the supply of metabolizable lysine, thus maximizing milk and component production. However, to effectively incorporate these products in a diet, it is essential to understand their feed stability, rumen stability, intestinal release and the biological response they elicit in the cow.

Accurately estimating the AA availability of any rumen protected amino acid (RP-AA) used in dairy rations is an extremely important yet challenging task. With numerous methodologies used in the industry today, it can be confusing and difficult to know which techniques yield the most accurate results. *In vivo* stable isotope approaches have been shown to assess individual amino acid absorption from both RP-AA and feed ingredients with adequate precision and accuracy (Estes et al., 2018; Rebelo and Lee, 2024).

Isotopes are elements that contain the same number of protons, but different number of neutrons in their nuclei, so they differ slightly in their atomic mass. Stable isotopes (such as carbon 13 [13C] and nitrogen 15 [15N]) are popular in both nutritional and environmental research because they have the advantage of generally behaving exactly as their non-isotopic nutrient or metabolite that is of interest, but do not emit harmful radiation like their radioactive isotope counterparts.

Once the metabolizable lysine content of a nutrient has been accurately established, the milk and component responses can be assessed when supplementing diets with specific levels of the nutrient using controlled trials.

This paper will discuss the results of an experiment using a stable isotope technique to determine the bioavailability of Balchem's newly developed RP-Lys product, AminoShure-XL. Three production trials designed to determine the impact of supplementing AminoShure-XL on milk and component production in dairy cows are also summarized in this document.

Material and Methods

Study 1 was conducted as a 6 × 8 Youden Square design with 8 periods and 8 treatments. Study 3 and 4 were conducted as a 5×5 Latin square design with 5 periods each lasting 14 days. The first 10 days of each period were for treatment adaptation and the last four days were for sampling. Study 5 was conducted as a 5×10 Latin rectangle design with 5 periods each lasting 18 days. The first 14 days of each period were for treatment adaptation and the last four days were for sampling. In all experiments, cows were housed in free stall barns equipped with a calan gate feeding system to allow quantification of individual feed intake.

Study 1: Fernandes et al. (2024)

Six Holstein heifers were enrolled in a 6 x 8 Youden Square design with 8 periods and 8 treatments. One common base ration predicted to be sufficient in metabolizable methionine (MP-Met) and metabolizable lysine (MP-Lys) using the NASEM (2021) was fed to all cows. The trial consisted of the base diet supplemented with one of 8 different RP-AA treatments, but only the AminoShure-XL treatment will be discussed. For the first 10 d of each 14-day period, heifers were fed daily. During the last 4 days of each period, heifers were fed every 2 hrs at 95% ad libitum to achieve steady state conditions. AminoShure-XL was supplemented at a rate of 118.5 g/h/d (66.4 g/h/d of Lys; 30% above diet supply).

On the last day of each period, a saline solution containing U-[13C]-labelled AA was infused into the jugular vein of each heifer for 12 hours and blood samples were collected. Isotopic enrichment of plasma AA in response to the jugular infusion was then measured and modelled in a 3-pool model (which encompasses protein turnover pools) to derive AA plasma absorption rates. Bioavailability was then calculated assuming a 7% loss of AA during first pass through the splanchnic tissues.

Study 2: Estes et al. (2024)

In this experiment, six RP-Lys products were evaluated for TMR stability: USA Lysine® (Kemin® Industries; 51.2% Lys), LysiGem® (Kemin® Industries; 55% Lys), AjiPro®-L (Ajinomoto®; 40% Lys), AminoShure®-XL (Balchem® Corporation; 54.4% Lys), Smartamine® ML (Adisseo; 44% Lys) and ReLys® (Vetagro®; 33% Lys). A negative control (no RP-Lys added) as well as a positive control (unprotected Lys HCl) were tested to ensure complete recovery of any potential free lysine released from the RP-Lys products.

A corn silage and haylage-based TMR (DM = 36.4%; pH = 4.6) was used in the experiment. For each treatment, the TMR (200 g) and the equivalent of 1 g of Lys from each product (RP-Lys or unprotected Lys) were weighed in triplicate and gently mixed for 30 seconds in a sealable ziplock bag.

The controls and RP-Lys treated TMRs were stored unsealed for 0, 6, 12 and 24 hr at 22°C (72°F). At each timepoint, the contents of each Ziplock bag were transferred to a strainer bag (250 µM pore size) and shaken for 1 min with 1 L of distilled water to facilitate solubilization of any free Lys released from the products. A sample of the solution was then taken, filtered through a 0.45µM disk filter and submitted to a third-party laboratory (Agriculture Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO) for free lysine analysis.

Study 3: Barnard et al. (2016)

Ten multiparous Holstein cows in peak to mid-lactation at the start of the experiment (100 DIM) were enrolled in each square. One common base ration predicted

to be deficient in MP-Lys (-14.5 g/d), but sufficient in NRC (2001) energy requirements using the Cornell Net Carbohydrate and Protein System (version 6.55) was fed to all cows daily to achieve 5-10% refusals. The trial consisted of the base diet supplemented with one of three different AminoShure-XL prototypes, but only the negative control (NC, no RP-Lys), positive control (NC supplemented with blood meal, BM) and a single AminoShure- XL prototype which exhibited the highest bioavailability of the three prototypes in the study will be discussed. This prototype was an early predecessor to the AminoShure-XL version that has been commercialized.

Study 4: Fleming et al. (2019)

To more accurately characterize the bioavailability of AminoShure-XL, a dose titration trial was conducted with an early version of AminoShure-XL. Ten multiparous Holstein cows in peak to mid-lactation at the start of the experiment (85 ± 6 DIM) were enrolled in each square. One common base ration predicted to be deficient in MP-Lys (-16.1 g/d) but sufficient in NRC (2001) energy requirements using the Cornell Net Carbohydrate and Protein System (version 6.55) was fed to all cows daily to achieve 10% refusals. The trial consisted of the base diet (NC) supplemented with either AjiPro-L (AL) or the AminoShure-XL (XL) prototype supplemented at 55% (XL 55% dose rate), 78% (XL 78% dose rate) or 102% (XL 102% dose rate) of the Lys content provided by AjiPro-L.

Study 5: Bailey et al. (2019)

To further characterize the bioavailability of AminoShure-XL, a second dose titration trial was conducted with the commercial version of AminoShure-XL. Ten multiparous Holstein cows in peak to mid-lactation at the start of the experiment (94 +/- 25 DIM) were enrolled in a 5 x 10 Latin rectangle design with 5 periods each lasting 18 days. The first 14 days of each period were for treatment adaptation and the last four days were for sampling. Cows were housed in a free stall barn equipped with a calan gate feeding system to allow quantification of individual feed intake. One common base ration predicted to be deficient in MP-Lys (-16.5 g/d) but sufficient in NRC (2001) energy requirements using the Cornell Net Carbohydrate and Protein System (version 6.55) were fed to all cows daily to achieve 5-10% refusals. The trial consisted of the base diet (NC) supplemented with either AjiPro-L or AminoShure-XL supplemented at 55% (XL 55% dose rate), 71% (XL 71% dose rate) or 100% (XL 100% dose rate) of the Lys content provided by AjiPro-L.

For studies 3-5, all diets were supplemented with RP-Met to ensure that there was no Met deficiency in any of the treatments. Milk samples were collected at each milking (AM and PM) on the last four days of each period and were sent to DHIA (Virginia Tech and University of Tennessee trials) or Dairy One (University of Delaware trial) for milk component analysis. Milk yields were also recorded at each milking.

Results and Discussion

Study 1. Fernandes et al. (2024)

Based on the isotopic enrichment and subsequent modelling work, the Lys plasma appearance coming from AminoShure-XL was 59.8%. Once corrected for Lys loss during first pass use, the subsequent bioavailability of AminoShure-XL was 64.0% (Table 1).

Table 1. **AminoShure-XL bioavailability results**

	Bioavailability	Metabolizable Lysine
AminoShure-XL	64.0%	34.6%

Study 2: Estes et al. (2024)

The TMR stability observed in this study varied greatly among the RP-Lysine products (Figure 1).

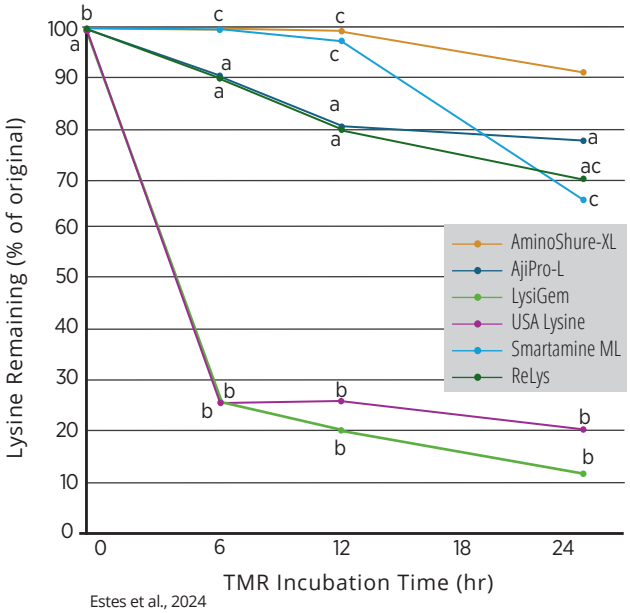
LysiGem and USA Lysine behaved similarly at all incubation times and experienced the most damage of all the products tested. At 6 hours of TMR exposure, only 25% of the Lys content in LysiGem and USA Lysine was remaining. As exposure time increased, both products continued to degrade with only 13% and 20% of their Lys content remaining at 24 hr of TMR incubation, respectively.

Smartamine ML remained stable with minimal damage through 12 hr of TMR exposure. However, after 24 hr in the TMR, the product succumbed to the environmental challenge, resulting in a Lys stability value of 65%.

Both AjiPro-L and ReLys experienced a more linear stability pattern across all timepoints, with roughly 99%, 89%, 80% and 70-77% of its Lys content remaining at 0, 6, 12 and 24 hrs of TMR exposure, respectively.

AminoShure-XL was consistently the most TMR stable product with 91% Lys remaining at the conclusion of the 24 hour experiment.

Figure 1. **TMR Stability of RP - Lysine Products**



Estes et al., 2024

[abcd]Values with differing superscripts within a timepoint are considered statistically different (P<0.05).

Study 3. Barnard et al. (2016)

In the University of Delaware experiment (Table 2 and Figure 2), the AminoShure-XL prototype significantly increased milk yield over the negative control by 4.3 lbs/d (1.95 kg/d). The AminoShure-XL prototype and the blood meal positive control treatment were not statistically different in terms of milk yield. Dry matter intake as well as milk protein and casein concentrations/yields were unaffected by treatment.

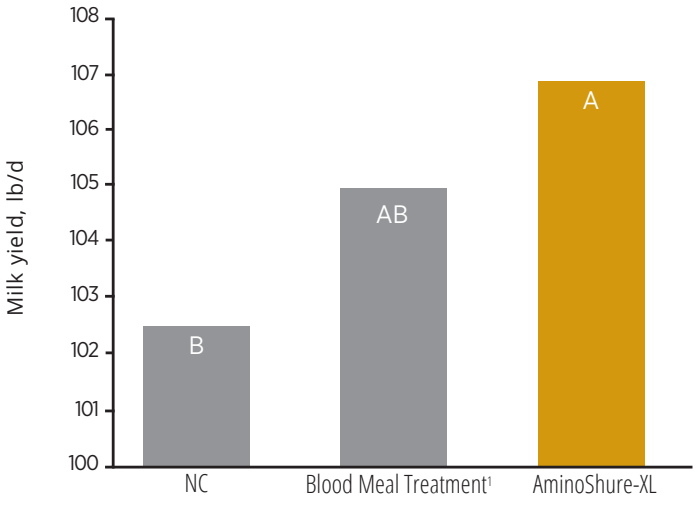
Table 2. **Effects of supplemental RP-Lys on milk yield and composition in lactating dairy cows at the University of Delaware**

	Treatment[1]				
	NC	Blood Meal	AminoShure-XL	SEM	*p*-Value
DMI, lbs/d	60.59	60.98	59.55	1.37	0.51
Milk,lbs/d	**102.40^{B}**	**104.87$^{A, B}$**	**106.70^{A}**	**1.87**	**0.02**
Fat, %	**3.57^{X}**	**3.41^{Y}**	**3.57^{X}**	**0.06**	**0.06**
Fat, lbs/d	**3.64$^{A, B}$**	**3.53^{C}**	**3.70^{A}**	**0.04**	**0.03**
Protein, %	2.92	2.90	2.92	0.02	0.21
Protein, lbs/d	3.02	2.98	3.00	0.02	0.11
Casein, %	2.35	2.34	2.36	0.01	0.18
Casein, lbs/d	2.42	2.40	2.42	0.02	0.20

Barnard et al., 2016

[a, b]Means with different superscript letters within the same row are significantly different (P < 0.05).
[x, y]Means with different superscript letters within the same row are considered a trend (0.05 ≤ P ≤ 0.1).
[1]NC = Negative control diet; Blood Meal = NC supplemented with blood meal; AminoShure-XL = NC supplemented with AminoShure-XL.

Figure 2. **Effects of supplemental RP-Lys on milk yield in lactating dairy cows at the University of Delaware**



Barnard et al., 2016

[a, b]Treatments with different letters are significantly different (P < 0.05).
[1]NC = Negative control diet; Blood Meal = NC supplemented with blood meal; AminoShure-XL = NC supplemented with AminoShure-XL.

Study 4. Fleming et al. (2019)

The milk protein response from the Virginia Tech experiment is shown in Table 3 and Figure 3. While there was no statistical increase in milk protein concentration with the addition of AjiPro-L to the diet, there was a significant linear increase in response to feeding increasing levels of the AminoShure-XL. For every 1 g of Lys fed from the AminoShure-XL, there was a .002% increase in milk protein concentration. No differences in DMI or milk yield were observed across treatments. Though not statistically different, milk protein yield numerically increased with increasing AminoShure-XL dose (3.46 lbs, 3.50 lbs, 3.54 lbs for the low, medium and high dose, respectively).

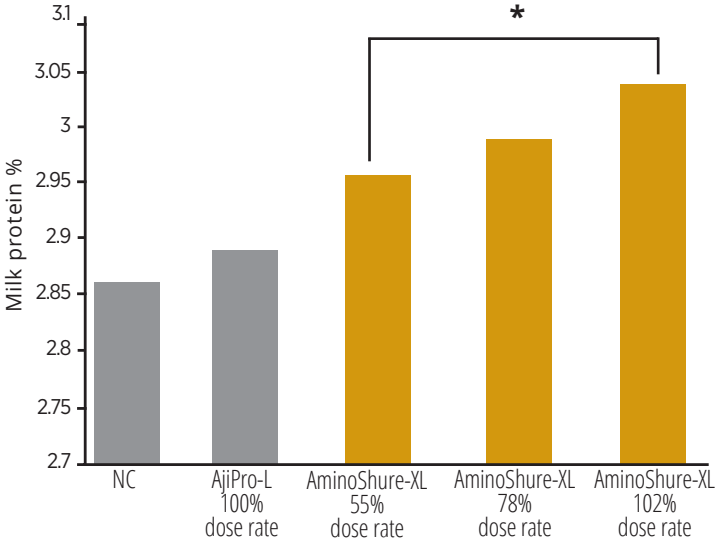
Table 3. **Effects of supplemental RP-Lys on milk yield and composition in lactating dairy cows at Virginia Tech**

	Treatment[1]					
	NC	AjiPro-L 100% dose rate	AminoShure-XL 55% dose rate	AminoShure-XL 78% dose rate	AminoShure-XL 102% dose rate	AminoShure-XL dose *p*-Value
DMI, lbs/d	60.98	59.95	61.33	61.49	61.64	0.87
Milk, lbs/d	117.15	117.69	117.24	117.28	117.32	0.96
Protein, %	**2.86**	**2.89**	**2.95**	**2.99**	**3.03**	**0.02**
Protein, lbs/d	3.37	3.40	3.46	3.50	3.54	0.30

Fleming et al., 2019

[1]NC = Negative control diet; AjiPro-L = NC supplemented with AjiPro-L; AminoShure-XL 55% dose rate = NC supplemented with AminoShure-XL at 55% of the Lys content of AjiPro-L; AminoShure-XL 78% dose rate = NC supplemented with AminoShure-XL at 78% of the Lys content of AjiPro-L; AminoShure-XL 102% dose rate = NC supplemented with AminoShure-XL at 102% of the Lys content of AjiPro-L.

Figure 3. **Effects of supplemental RP-Lys on milk protein concentration in lactating dairy cows at Virginia Tech**



Fleming et al., 2019

*Significant linear dose response (P = 0.025). For every 1 g of Lys fed from AminoShure-XL = 0.002% increase in milk protein (%).

[1]NC = Negative control diet; AjiPro-L = NC supplemented with AjiPro-L; AminoShure-XL 55% dose rate = NC supplemented with AminoShure-XL at 55% of the Lys content of AjiPro-L; AminoShure-XL 78% dose rate = NC supplemented with AminoShure-XL at 78% of the Lys content of AjiPro-L; AminoShure-XL 102% dose rate = NC supplemented with AminoShure-XL at 102% of the Lys content of AjiPro-L.

Study 5. Bailey et al. (2019)

The University of Tennessee trial also showed that supplementing increasing levels of AminoShure-XL resulted in a significant dose response (Table 4 and Figure 4). In these experimental conditions, an optimum dose of 17.9 g/d of Lys supplied from AminoShure-XL promoted an overall benefit of 3.3 lb/d (1.51 kg/d; P = 0.03). There was no statistical increase in milk yield with the addition of AjiPro-L to the NC diet. Additionally, supplementation of AminoShure-XL resulted in a quadratic trend (P = 0.07) for milk protein concentration were increasing the dose of the AminoShure-XL from low to medium to high resulted in a milk protein response of 3.10, 3.12 and 3.15%, respectively. No differences in DMI, milk fat concentration/yield or milk protein yield were observed across treatments.

Table 4. **Effects of supplemental RP-Lys on milk yield and composition in lactating dairy cows at the University of Tennessee**

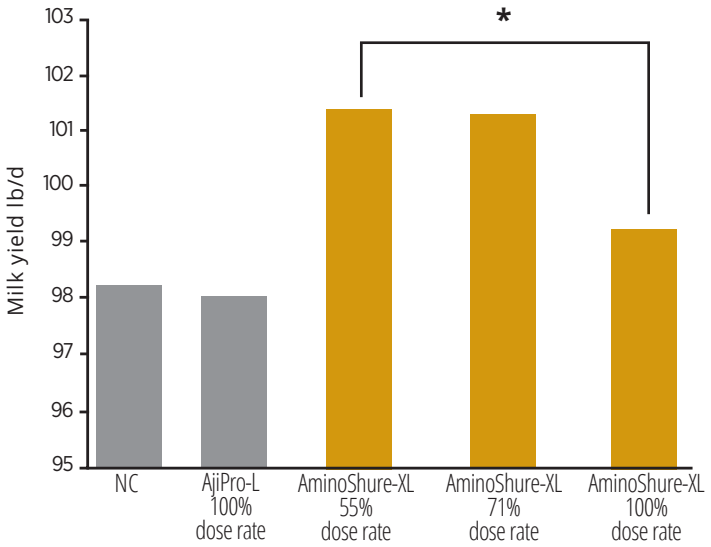
	Treatment[1]						
	NC	AjiPro-L	AminoShure-XL 55% dose rate	AminoShure-XL 71% dose rate	AminoShure-XL 100% dose rate	SEM	AminoShure-XL dose *p*-Value*
DMI, lbs/d	65.7	65.2	66.3	64.8	65.9	1.7	-
Milk, lbs/d	**98.1**	**97.9**	**101.4**	**101.4**	**99.2**	**2.6**	**0.03**
Protein, %	**3.15**	**3.12**	**3.10**	**3.12**	**3.15**	**0.03**	**0.07**
Protein, lbs/d	3.06	3.02	3.15	3.15	3.09	0.09	NS
Fat, %	4.44	4.40	4.34	4.18	4.27	0.16	NS
Fat, lbs/d	4.21	4.21	4.43	4.30	4.25	0.22	NS

Bailey et al., 2019

[1]NC = Negative control diet; AjiPro-L = NC supplemented with AjiPro-L; AminoShure XL 55% dose rate = NC supplemented with AminoShure-XL at 55% of the Lys content of AjiPro-L; AminoShure-XL 71% dose rate = NC supplemented with AminoShure-XL at 71% of the Lys content of AjiPro-L; AminoShure-XL 100% dose rate = NC supplemented with AminoShure-XL at 100% of the Lys content of AjiPro-L.

*NS = Not significantly different from zero (P > 0.1).

Figure 4. **Effects of supplemental RP-Lys on milk yield in lactating dairy cows at the University of Tennessee**



Bailey et al., 2019

*Significant dose response (P = 0.03). In this experimental condition, an optimum dose of 17.9 g/d of Lys supplied from AminoShure-XL promoted an overall benefit of 3.3 lb/d (1.51 kg/d).

[1]NC = Negative control diet; AjiPro-L = NC supplemented with AjiPro-L; AminoShure XL 55% dose rate = NC supplemented with AminoShure-XL at 55% of the Lys content of AjiPro-L; AminoShure-XL 71% dose rate = NC supplemented with AminoShure-XL at 71% of the Lys content of AjiPro-L; AminoShure-XL 100% dose rate = NC supplemented with AminoShure-XL at 100% of the Lys content of AjiPro-L.

Summary

In vivo stable isotope approaches offer both precision and accuracy for determining the bioavailability of RP-AA. Using the isotopic enrichment of plasma and subsequent modelling/calculations, the bioavailability of AminoShure-XL was determined to be 64%.

Vast differences in TMR stability were noted across the different RP-Lys products. These findings highlight that TMR stability must be accounted for as damage to the protective coating of these products may occur prior to consumption by the cow, potentially reducing the efficacy of RP-Lys products. Of all the products tested, AminoShure-XL was consistently the most TMR stable across all timepoints, experiencing greater than 90% stability at any exposure time.

Based on these production experiments, AminoShure-XL has proven to deliver a consistent and reliable supply of MP-Lys, as evidenced by the impressive dose responses observed in both milk yield and protein concentration. When compared to blood meal, AminoShure-XL not only increased fat concentration and yield, but also maintained a similar milk yield response. This consistency in response further underscores the product's superiority as a source of MP-Lys. Furthermore, AminoShure-XL outperformed AjiPro-L, showing greater improvements in milk and component responses when supplemented at 55%, 75%, or 100% of the Lys content of AjiPro-L. This clearly demonstrates the advantages of Balchem's advanced encapsulation technology.

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