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# M311

# Determining the Bioavailability of Lysine in AjiPro-L Using the Plasma-Free Amino Acid Dose Response Method

N.L. Whitehouse *[1], E. S. Fletcher[1], A.F. Brito[1], C. G. Schwab[2], and I. Shinzato[3]. [1]University of New Hampshire, [2]Schwab Consulting, LLC, and [3]Ajinomoto Heartland, Inc.

## INTRODUCTION

It is well documented that Lys and Met are usually the first two limiting AA for lactating dairy cows (NRC, 2001). To help achieve optimal concentrations of Lys in MP, a number of rumen-protected Lys (RP-Lys) supplements have been introduced to the market. Some that are available in the United States are Megamine-L® (Arm and Hammer), LysiPEARL™ (Kemin), Lysine 35™ (Jefo), AjiPro™ -L (Ajinomoto), MetaboLys® (H.J. Baker), and USA Lysine™ (Land O' Lakes). These products are a welcomed addition to dairy cattle nutrition with the expectation that they can be an alternative, or at least a partial substitute, for high Lys protein supplements like blood, fish, and soya proteins. However, fundamental to their use is having reliable estimates of Lys bioavailability and comparative assessments of efficacy. While companies provide estimates of Lys bioavailability for their products, the data is usually based on different techniques which gives limited insight about how the products compare in efficacy and cost-effectiveness.

The plasma free Lys dose-response approach is an attractive approach for obtaining estimates of Lys bioavailability of RP-Lys supplements because it has the potential to provide animal-derived estimates of bioavailability under conditions similar to commercial use. The approach assumes that a positive linear relationship exists between plasma free Lys concentrations and increasing amounts of absorbed Lys. This relationship has been confirmed by several research groups by continuous infusions of graded amounts of Lys-HCL into the abomasum or duodenum (King et al., 1991; Guinard and Rulquin, 1994; McLaughlin, 2002; Rulquin and Kowalczyk, 2003; Castro et al., 2008; Hanigan et al., 2009; Whitehouse et al., unpublished). In these experiments, the number of infusion treatments varied from four to seven and daily dosages varied from 0 to 40, 0 to 63, 0 to 66, 0 to 75, 0 to 84 and 0 to 180 g/cow per day. The linearity of response has been observed in cows fed both Lys-deficient diets (Guinard and Rulquin, 1994, King et al., 1991; McLaughlin, 2002; Whitehouse et al., unpublished) and Lys adequate diets (Castro et al., 2008; Hanigan et al., 2009; Rulquin and Kowalczyk, 2003; Whitehouse et al., unpublished).

Using the highly protected L-arginine (HPArg) procedure (Nocek et al., 2010, Robinson et al., 2011), Ajinomoto has concluded that AjiPro-L has a 40% bioavailability. AjiPro-L consists of a matrix core of L-Lys monohydrochloride and hydrogenated fat covered with a protective layer of vegetable fat.

## OBJECTIVE

To further verify if the plasma free Lys dose-response could be effective to determine Lys bioavailability of RP-Lys products, the objective of this experiment was to use the method to obtain an estimate of the bioavailability of Lys in AjiPro-L using lactating Holstein cows.

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## MATERIALS AND METHODS

- Six multiparous Holstein cows (90 to 130 DIM) equipped with ruminal cannulas were assigned to a 6 x 6 Latin square with 7-day experimental periods.
- Infusion lines were placed into the omasum using the procedure of Gressley et al. (2006).
- Treatments were (g/22.7 kg DMI): 1) 0 (negative control), 2) 30 infused, 3) 30 fed, 4) 45 fed, 5) 60 infused, and 6) 60 fed. Cows were fed ad libitum. Infused and fed treatments were adjusted every week according to feed intake.
- Infusions were continuous except for 45 min twice a day when cows were moved from their stalls for milking.
- To reduce the chance of "carry-over effects" of previous treatments, treatments were not administered on day-1 of periods 2-6.
- Cows were fed a Lys-adequate diet 3 times daily (0500, 1400, and 2100 h) (Table 1) with one third of their daily allotted TMR offered at each feeding.
- To ensure complete consumption, AjiPro-L was mixed with approximately 456 g of the energy mix (steam flaked corn, citrus pulp, corn meal, soy hulls, and molasses) in 6.5-gal feed tubs and placed in front of the cows 1 h before each feeding. After 5-10 min, 250 g of TMR was added to the feed tub and reintroduced to the cows to help ensure that the entire product was consumed. In a few occasions when the feed was not completely consumed within 20 min, it was placed into the rumen via the rumen cannula.
- Milk samples were taken at each milking the last 3 days of each experimental period.
- Blood samples were taken in the last 3 days of each experimental period at 2, 4, 6, and 8 h after the 0500-h feeding. Samples were obtained from the tail vein using 10-mL vacutainer tubes containing 15% EDTA. Blood samples were centrifuged within 15 min of sampling at 3,300 x g for 20 min at 5°C. Plasma was deproteinized with 15% sulfosalicylic acid and recentrifuged at 3,300 x g for 20 min at 5°C. Deproteinized plasma was stored at -80°C for later AA analysis.
- The 4 plasma samples for each day were composited into 1 daily sample/cow.
- The UNIVARIATE procedure of SAS 9.2 was used to determine outlier values. Observations greater than 2.5 standard deviations from the mean for plasma Lys as a % of total AA (TAA) were considered outliers. Plasma AA data was analyzed using the MIXED procedure of SAS 9.2. Linear, quadratic, and cubic effects were determined using the CONTRAST statement of SAS 9.2. Significance was noted at $P < 0.05$. The LSMEANS for Lys generated from the MIXED procedure were subjected to the PROC REG procedure of SAS 9.2 to generate the linear regression lines, slopes, and $r^2$.
- The bioavailability of Lys was calculated using the slope ratio equation. Plasma free Lys concentrations were expressed both as ug/mL and uM before expression as a % of total AA and regression analysis.

**[Table 1] Ingredient composition of the ration.**

| Ingredient | % DM |
|---|---|
| Corn silage, mature | 36.56 |
| Mixed mostly grass silage, mid-maturity | 13.72 |
| Alfalfa hay | 2.70 |
| Steam flaked corn | 4.25 |
| Citrus pulp | 8.07 |
| Corn meal | 11.03 |
| Soy hulls | 3.39 |
| Distillers grains | 0.89 |
| Soybean meal | 8.43 |
| Canola meal | 2.67 |
| ProvAAL Elite1 | 2.20 |
| Molasses | 1.19 |
| Urea | 0.32 |
| BergaFat | 1.15 |
| Mineral/vitamin mix | 3.44 |

[1]Contains blood meal, feather meal, HMB, and 2.75% Smartamine® M.

**[Table 2] NRC (2001) evaluation of the basal formulated diet[1] based on a mean.**

| | | | |
|---|---|---|---|
| NDF, % DM | 33.3 | $NE_L$ allowable milk, kg/d | 34.8 |
| Forage NDF, % DM | 24.8 | MP allowable milk, kg/d | 35.1 |
| ADF, % DM | 21.8 | Actual milk, kg/d | 41.3 |
| NFC, % DM | 42.6 | | |
| ME, Mcal/kg DM | 2.53 | CP, % DM | 15.9 |
| NEL, Mcal/kg DM | 1.61 | RDP, % DM | 9.8 |
| EE, % DM | 4.3 | RUP, % DM | 6.0 |
| DCAD, meq/kg | 159 | | |
| | | RDP balance, g/d | -40 |
| NEL required, Mcal/d | 39.4 | RUP balance, g/d | -334 |
| NEL supplied, Mcal/d | 34.9 | | |
| NELbalance, Mcal/d | -4.5 | MP-bacterial, g/d | 1160 |
| | | MP-RUP, g/d | 1076 |
| MP required, g/d | 2612 | MP-endogenous, g/d | 102 |
| MP supplied, g/d | 2338 | | |
| MP balance, g/d | -274 | MP-Lys, % MP | 6.81 |
| | | MP-Met, % MP | 2.08 |
| DM intake-actual, kg/d | 21.7 | | |
| DM intake-predicted, kg/d | 26.2 | MP-Lys, g/d | 159 |
| | | MP-Met, g/d | 49 |

[1] Animal inputs were BW = 687 kg, DM intake = 21.7 kg/d, milk yield = 41.3 kg/d, milk fat = 3.56 %, milk true protein = 2.97 %.

## RESULTS

Increasing amounts of infused and fed Lys had no effect on milk yield, milk component concentrations or DMI (Table 3).

Lysine was the only plasma AA that increased linearly ($P < 0.05$) in response to infused or fed Lys (Figures 1 and 2; data not shown for other AA).

Expressing plasma Lys concentrations either as µg/mL or as µM, prior to expression as a % of TAA, resulted in similar regression equation parameters (Table 4).

**[Table 3] DM intake and milk yield and milk composition of cows without infused Lys, and NRC evaluation of basal diets (based on actual intake & production data).**

| Item | Lysine levels, g/d | | | | | Linear | Quadratic |
|---|---|---|---|---|---|---|---|
| | 0 | 30 | 45 | 60 | SE | P= | P= |
| **Milk yield, kg/d** | | | | | | | |
| Infusion | 40.6 | 40.2 | | 41.1 | 1.47 | 0.62 | 0.45 |
| AjiPro-L | 40.6 | 41.7 | 42.0 | 41.7 | 1.33 | 0.27 | 0.32 |
| **Fat, %** | | | | | | | |
| Infusion | 3.61 | 3.60 | | 3.60 | 0.092 | 0.97 | 0.98 |
| AjiPro-L | 3.61 | 3.51 | 3.51 | 3.52 | 0.053 | 0.32 | 0.31 |
| **Protein, %** | | | | | | | |
| Infusion | 2.96 | 3.01 | | 2.99 | 0.022 | 0.37 | 0.17 |
| AjiPro-L | 2.96 | 2.94 | 3.00 | 2.98 | 0.027 | 0.33 | 0.97 |
| **MUN, mg/dL** | | | | | | | |
| Infusion | 12.8 | 12.9 | | 12.8 | 0.62 | 0.99 | 0.92 |
| AjiPro-L | 12.8 | 13.1 | 13.0 | 13.2 | 0.76 | 0.67 | 0.93 |
| **DMI, kg/d** | | | | | | | |
| Infusion | 22.1 | 21.9 | | 22.0 | 1.04 | 0.88 | 0.88 |
| AjiPro-L | 22.1 | 21.4 | 20.9 | 21.7 | 0.80 | 0.35 | 0.18 |

Figure 1. Changes in Lys plasma concentrations with increasing amounts of fed or infused Lys expressed as % TAA on a ug/ml basis.

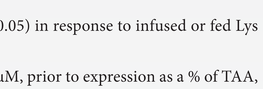
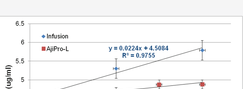



Figure 2. Changes in Lys plasma concentrations with increasing amounts of fed or infused Lys expressed as % TAA on a umole basis.

**[Table 4] Lysine "bioavailability" of AjiPro-L relative to that of infused Lys-HCl using changes in plasma Lys concentrations (Figures 1 and 2) as the response criteria.**

| Item | Infused Lys-HCL | | AjiPro-L | |
|---|---|---|---|---|
| | Slope | SE | Slope | SE |
| Slope for Lys in plasma | | | | |
| %TAA (ug/ml basis) | .0224 | .261 | .0078 | .123 |
| %TAA (ug/ml basis) | .0183 | .272 | .0068 | .097 |
| | Lys bioavailability relative to that of infused lysine-HCl[1] | | | |
| %TAA (ug/ml basis) | 34.8 | | | |
| %TAA (ug/ml basis) | 37.2 | | | |

[1]Calculated as slope for AjiPro-L divided by the slope for infused Lys x 100.




## DISCUSSION

The observed linear relationship between infused amounts of Lys and plasma free Lys concentrations is consistent with previous research (King et al., 1991; Guinard and Rulquin, 1994; McLaughlin, 2002; Rulquin and Kowalczyk, 2003; Castro et al., 2008; Hanigan et al., 2009; Whitehouse et al., unpublished).

Expressing plasma Lys concentrations as µg/mL, prior to expression as a % of TAA, yielded a Lys bioavailability estimate of 35% for AjiPro-L. Expressing plasma Lys concentrations as µM, prior to expression as a % of TAA, yielded a Lys bioavailability estimate of 37%. Both values are comparable to Ajinomoto's stated bioavailability of 40% and within the experimental error of determination.

## CONCLUSION

We conclude that the HPArg procedure, as described by Nocek et al. (2010) and Robinson et al. (2011), and the plasma free Lys dose response approach provide similar estimates of bioavailability of Lys from RP-Lys supplements.

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