# Assessing protected products for feed and ration formulation


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# Ruminant Lys and Met supplements in North America

## Lys supplements

- AjiPro® -L
- AminoShure-L
- Bovi-Lysine
- EB-Lys
- Lysine 35™
- LysiPEARL™
- Megamine-L™
- MetaboLys®
- NoviLys®
- Smartamine ML®
- USA Lysine™

## Met supplements

- Smartamine M®
- Mepron®
- AminoShure-M
- MetaboMet™
- MethioPlus™
- MetiPEARL™
- Novimet®
- EB-Met

RP-Met

- MetaSmart® (HMBi)
- Alimet®
- Rhodimet AT 88®
- MFP™ (CaMHA)

Met analogs

# Methods used for determining efficacy of RPAA supplements and procedural shortcomings

- ***In vitro methods***
  - Ammonia release
  - Amino acid release (modified 3-step method)
    - In rumen buffer
    - In abomasal buffer
    - In intestinal buffer
  - Amino acid release (Cornell/Ross assay)

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  - Amino acid release (Cornell/Ross assay)

No influence of animal effects

# Two examples of the modified 3-step in vitro approach for evaluating RPAA supplements

Miyazawa, Y., M. Miura, T. Fujieda, I. Shinzato, M.D. Stern and S.W. Fessenden. 2014. A three-step *in vitro* procedure for evaluating rumen-protected lysine products. Poster #M254, Abstract #1540 (Used a dissolution apparatus) (see poster)


Larson, H.E., I. Shinzato, M. Miura, I.J. Salfer, S. W. Fressenden, and M.D. Stern. 2015. Evaluation of three rumen-protected lysine sources produced in two different batches using a modified three-step *in vitro* procedure. Abstract #M422 (Used a media bottle system) (see poster)

The RPLs showed various characteristics; high/medium/low ruminal protection and high/low post-ruminal Lys release (Figure 1).

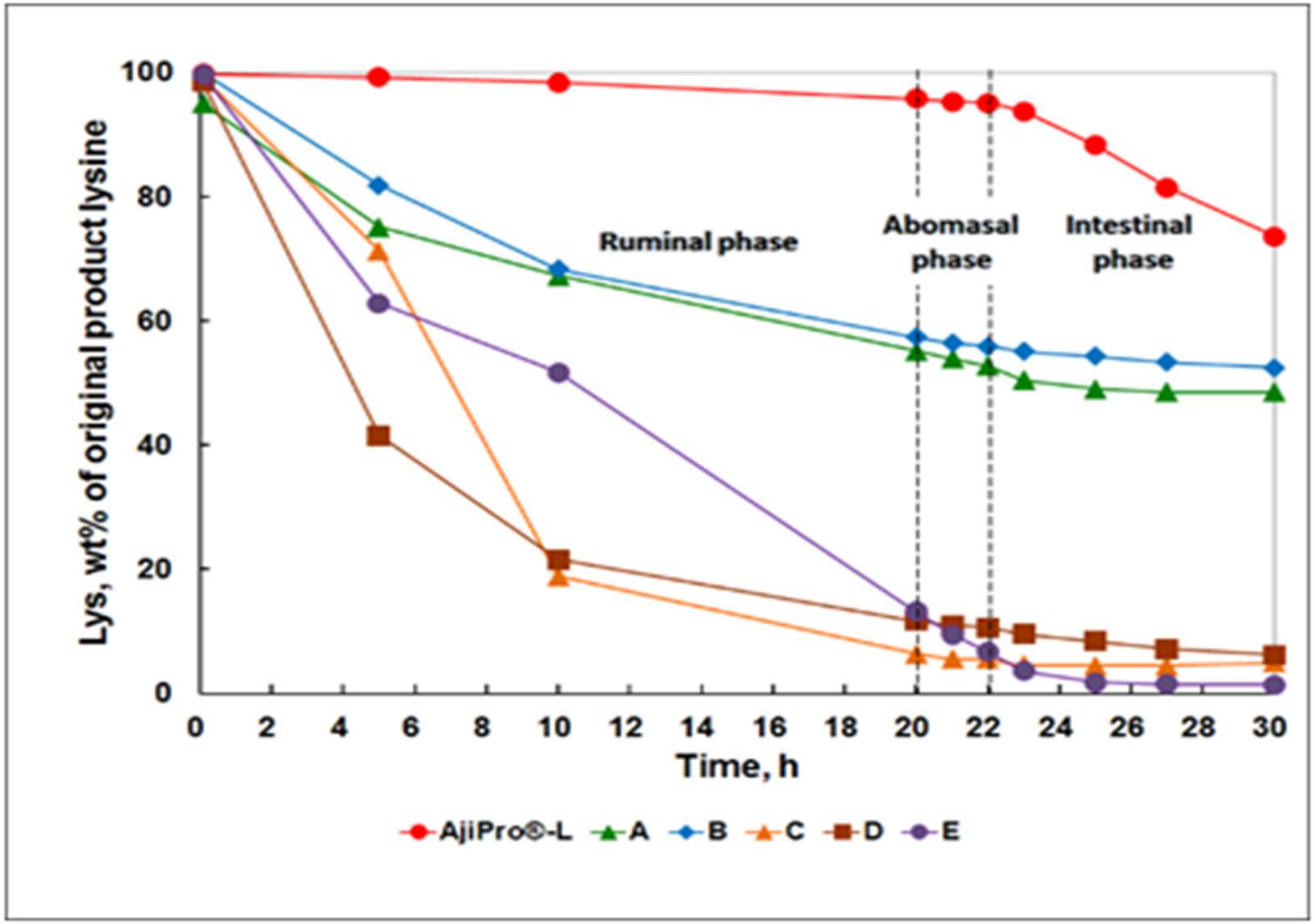


Figure 1. Three-step *in vitro* measurement of 6 RPLs.

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  - Ammonia release
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    - In rumen buffer
    - In abomasal buffer
    - In intestinal buffer
  - Amino acid release (Cornell/Ross assay)
- ***In situ methods***
  - Amino acid loss from rumen and intestinal bags

- Effects of eating & rumination not considered
- Disappearance from rumen bags means degradation
- Passage rates are needed
- Disappearance from mobile bags means absorption
- Products subjected to hindgut digestion
- Cannot be used with fine or soluble products

# In situ evaluation of Mepron® M85 in late lactation dairy cows


| | Hours of incubation in rumen | | | | |
|---|---|---|---|---|---|
| | 3 | 6 | 12 | 24 | 96 |
| | **Losses, %** | | | | |
| Rumen[1] | 6 | 8 | 22 | 38 | 87 |
| Postruminal[2] | 63 | 63 | 52 | 43 | 9 |
| Fecal[3] | 31 | 29 | 26 | 19 | 4 |
| | | | | | |
| Disappearance of Met entering small intestine | | | | | |
| | 67 | 68 | 66 | 70 | 70 |

[1] 0.75 g of product incubated in polyester bags.
[2] Incubated bags inserted in duodenal cannula and recovered in feces.
[3] percentage of initial Met recovered in feces.

**Overton et al. (1996)**

# *In situ* approach for measuring ruminal and postruminal losses of Met from Mepron® M85

**Exp. 1[1] (2000)**

| Ruminal incubation[1] (h) | Ruminal loss (%) |
|---|---|
| 0 | 7 |
| 1 | 4 |
| 2 | 9 |
| 4 | 19 |
| 8 | 32 |
| 10 | 33 |
| 12 | 37 |
| 16 | 46 |

**Exp. 2[2] (2000)**

| Lost in: | % of Met put into the bags |
|---|---|
| Rumen | 16 |
| Small intestine | 37 |
| Large intestine | 11 |
| Feces | 36 |
| Total | 100 |

**(2001)**

In situ trial involving 2 cows with duodenal and terminal ileum cannula

Top-dressed with 72 g/d

DM intake = 12.4 kg/d

66% bypassed rumen, 82% of that disappeared in small intestine

Disappeared in SI =54%

[1] 1.5 g of product was incubated in polyester bags in 3 mature cattle (1 steer and 2 non-lactating cows)
[2] Following rumen incubation for 4.5 h, bags were washed in water and then transferred into a pepsin-HCl solution (pH = 2.0) for 2.5 h to mimic abomasal digestion. Bags were then inserted into either duodenal or ileal cannula and recovered in the feces

**Berthiaume et al. (2000, 2001)**

# *In situ* and plasma Met responses to Mepron® M85

Rumen degradability = 22% [(A = 7%, B = 89%, C = 4%) $k_d$ of B = 2.3%, $k_p$ = 11%]

Intestinal disappearance (mobile bag) = 32%

Met bioavailability (78% x 0.32) = **25%**

Increase in plasma Met above control (0 g Mepron)

| 20 g Mepron | 63 g Mepron | 10.7 g infused Met |
|---|---|---|
| 33% | 66% | 59% |

Met bioavailability

[5.9 g of absorbed Met / (20 g x 0.87 Met)] = **34%**

[11.8 g of absorbed Met / (63 g x 0.87 Met)] = **22%**

Koenig and Rode (2001)

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    - In rumen buffer
    - In abomasal buffer
    - In intestinal buffer
  - Amino acid release (Cornell/Ross assay)
- ***In situ methods***
  - Amino acid loss from rumen and intestinal bags
- ***In vivo methods***
  - Milk protein dose-response approach (requires that cows always remain AA deficient)
  - Plasma free AA “area-under-the curve” (AUC) approach (requires feeding large amounts at one time)
  - Plasma free AA dose-response approach

# Use of the production dose-response approach at the University of New Hampshire

| Square | Met equivalents (g/25 kg DM intake) | | | | |
|---|---|---|---|---|---|
| Smartamine M | 0 | 10 | 15 | 20 | 25 |
| HMBi | 0 | 15 | 20 | 25 | 35 |
| HMB | 0 | 15 | 20 | 25 | 35 |

Schwab et al. (2001)

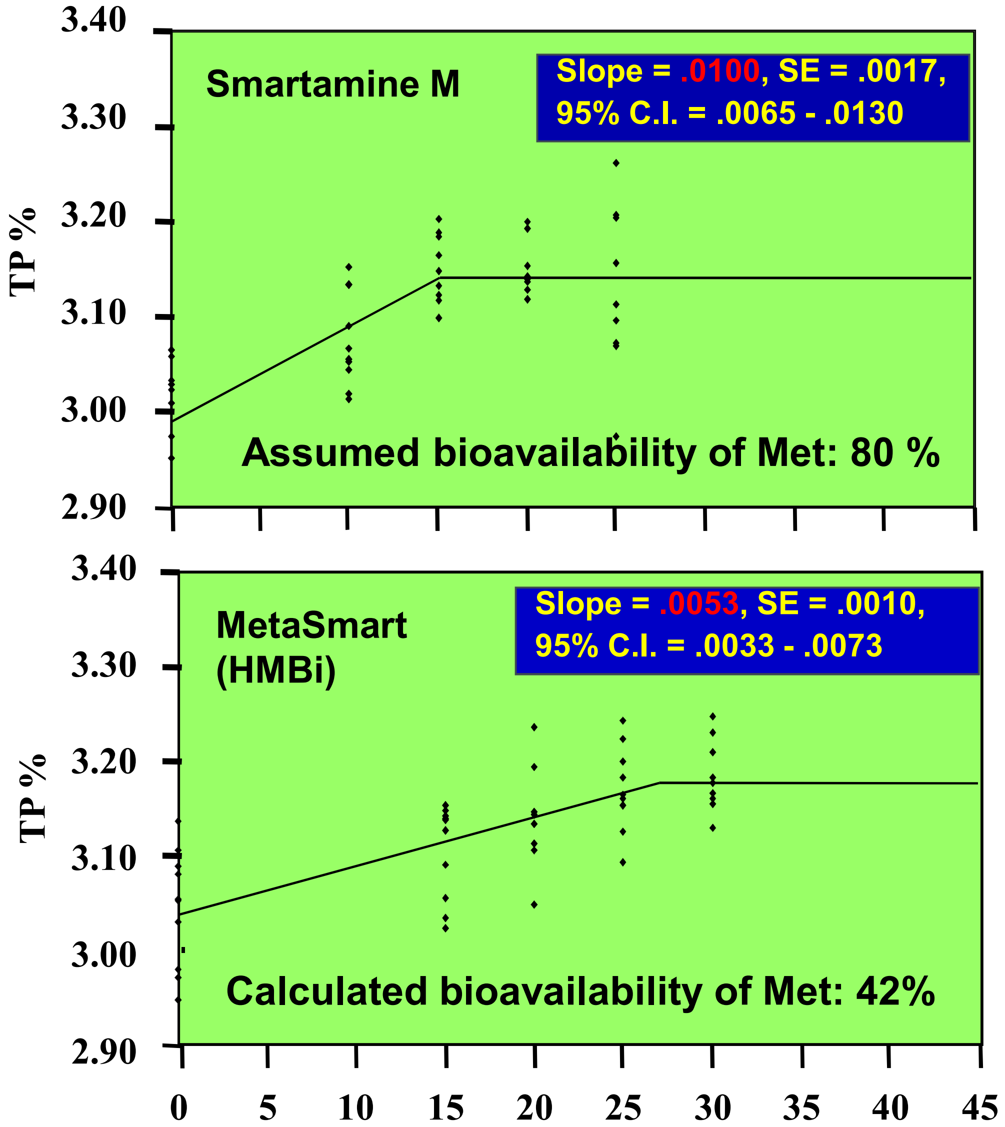
# Least square means for milk protein percentages

| Square | Treatment levels | | | | | $P$ = | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | L | Q |
| Smartamine | 2.99 | 3.08 | 3.14 | 3.15 | 3.13 | 0.00 | 0.01 |
| HMBi | 3.05 | 3.11 | 3.16 | 3.17 | 3.19 | 0.00 | 0.65 |
| HMB | 3.04 | 3.02 | 3.03 | 3.05 | 3.03 | 0.60 | 0.89 |

There were no effects ($P > 0.05$) of treatment levels on DMI (26.0 kg/d), milk yield (44.2 kg/d), milk fat content (3.51%) or MUN (11.3 mg/dL)

Schwab et al. (2001)

# Using the milk protein dose-response approach for estimating "Met-bioavailability" of MetaSmart and Rhodimet AT88

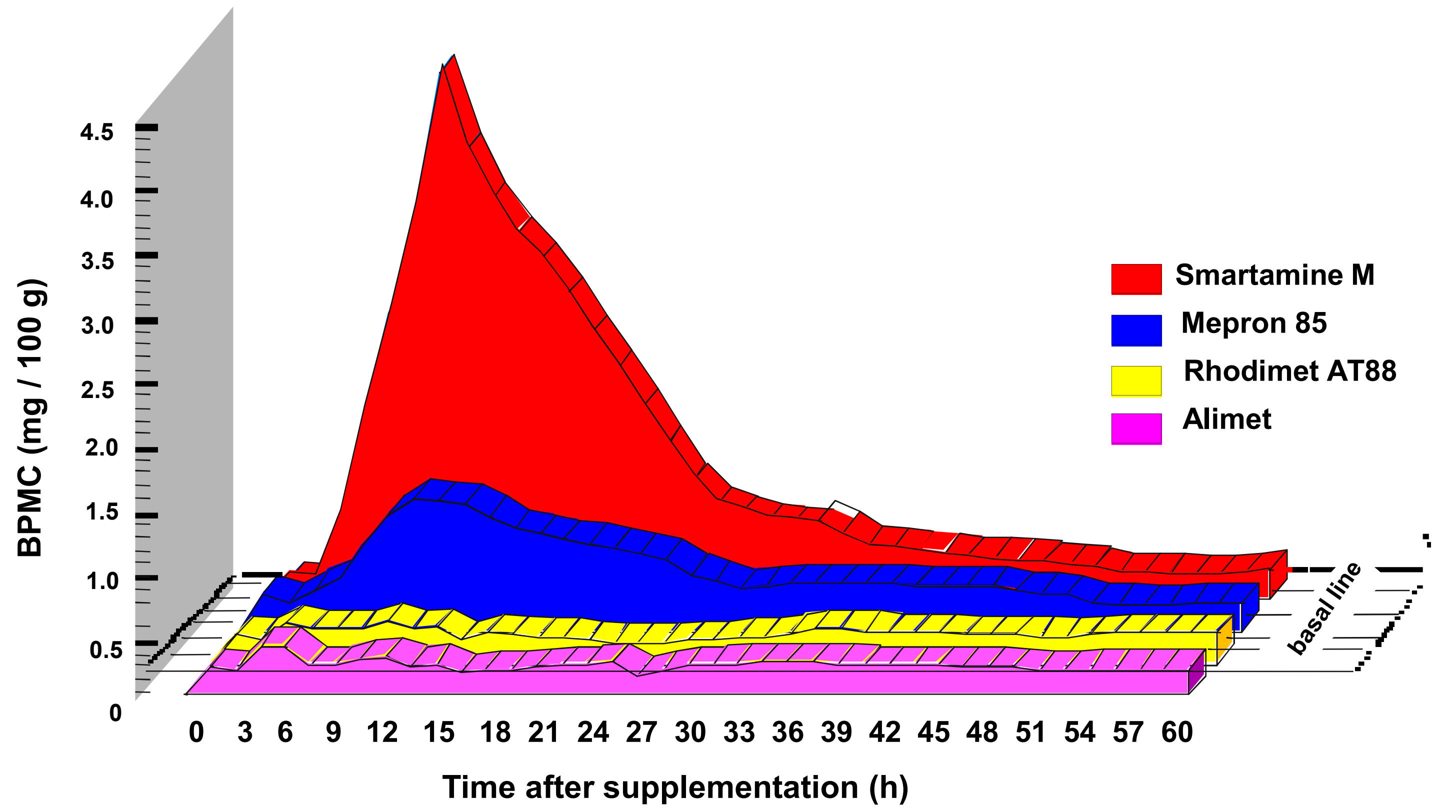



Notes:

1) Quantities in Met equivalents
2) Holstein cows
3) 40-45 kg milk

Schwab et al. (2001)

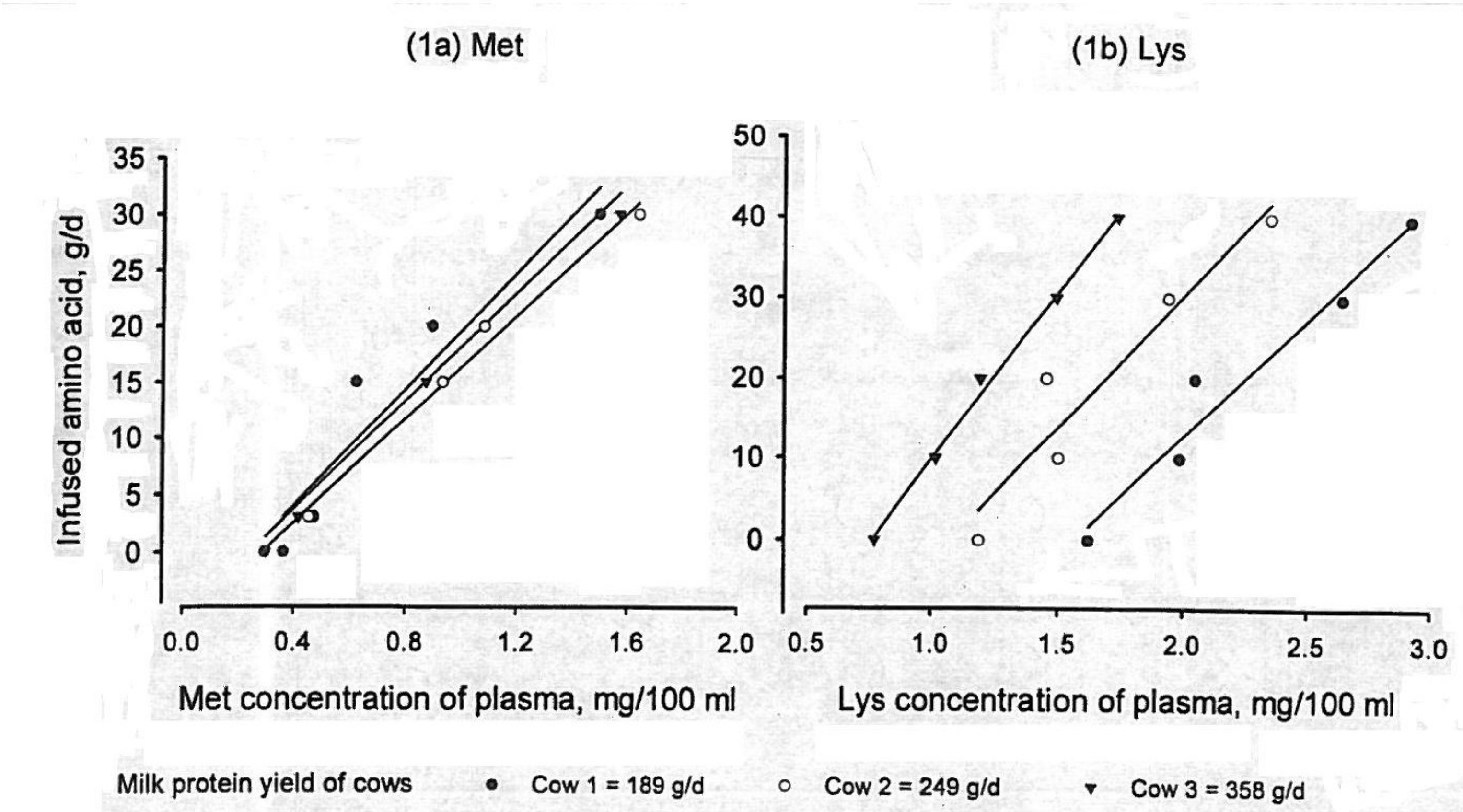
# Jugular plasma Met concentrations in cows given 50 g doses of two RPMet and two MHA products




The area-under-the-curve for Mepron M85 was 25% of Smartamine M and the two MHA products were 3.2 and 1.8% of Smartamine M.

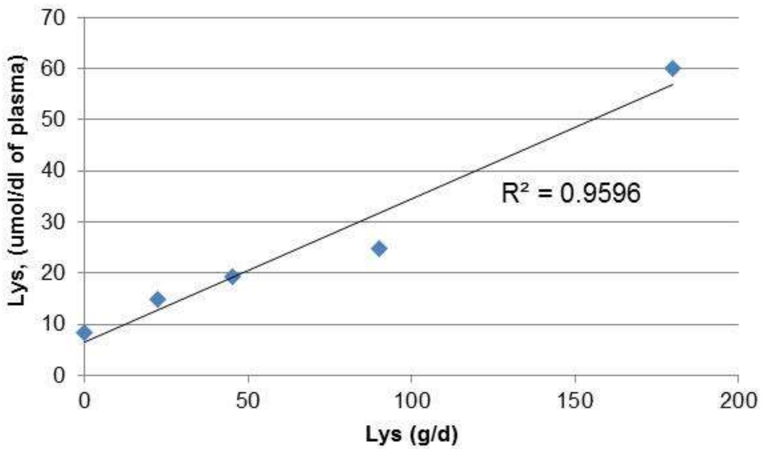
Robert et al. (1997)

# Plasma Met and Lys responses in low-yielding dairy cows to different levels of duodenally infused Met and Lys

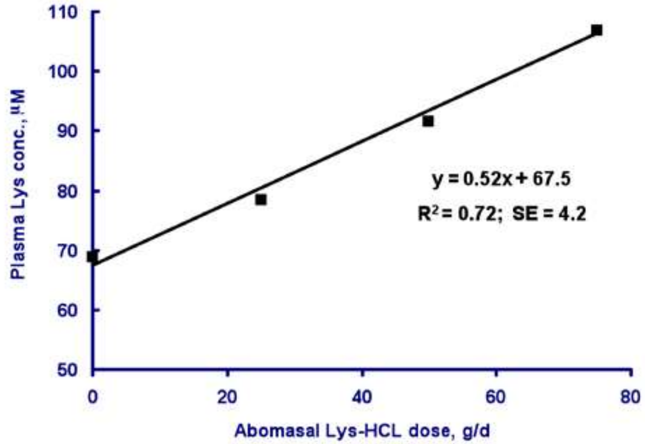



**Rulquin and Kowalczyk, 2003**

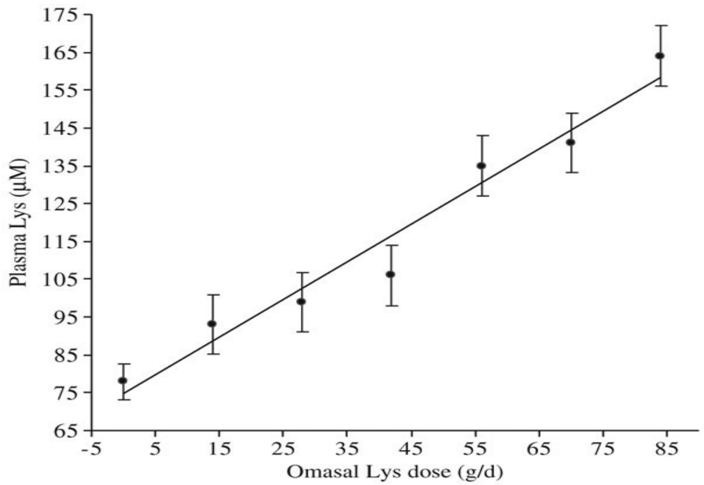
# Demonstrated linearity of response




King et al. (1991)




Hanigan et al. (2009)




Castro et al. (2008)

J. Dairy Sci. 100:9585-9601
https://doi.org/10.3168/jds.2017-12695

# The plasma free amino acid dose-response technique: A proposed methodology for determining lysine relative bioavailability of rumen-protected lysine supplements

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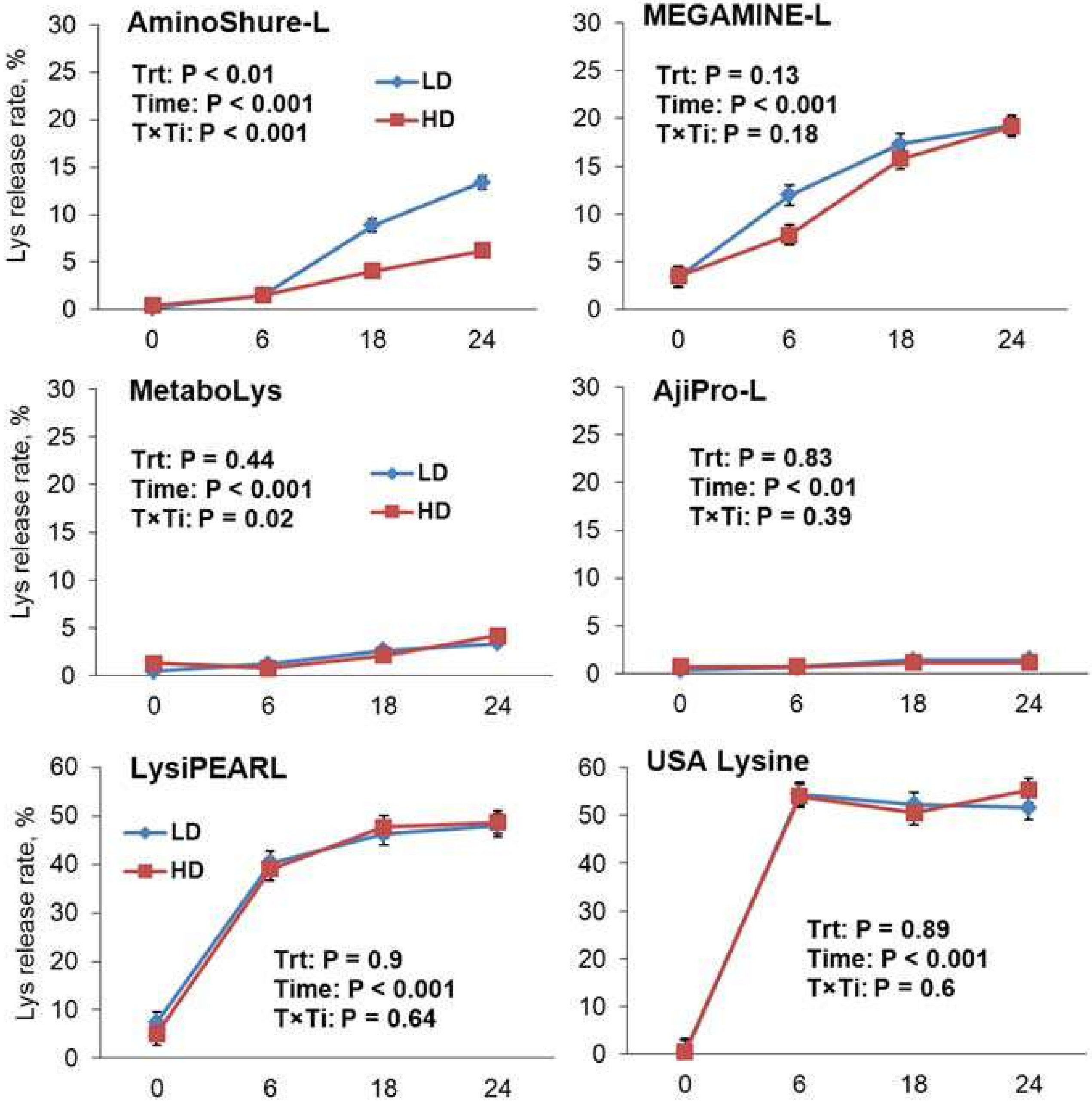
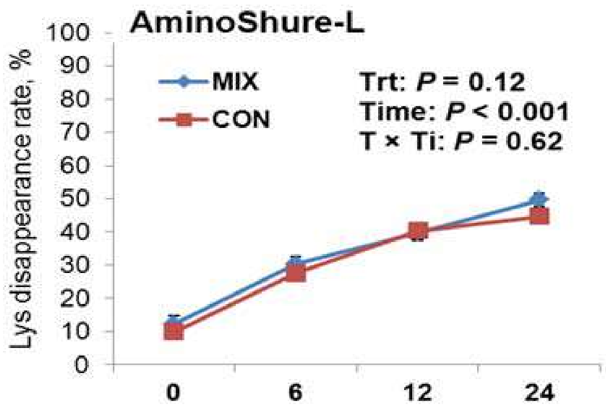
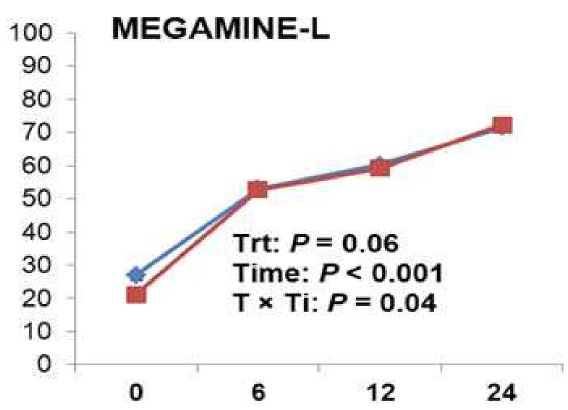
# University of New Hampshire approach for using the plasma free AA dose-response approach

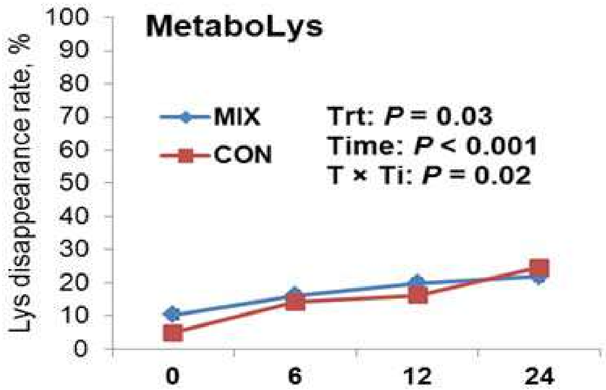
- High producing cows are fitted with ruminal cannula for abomasal infusion of the “unprotected” AA

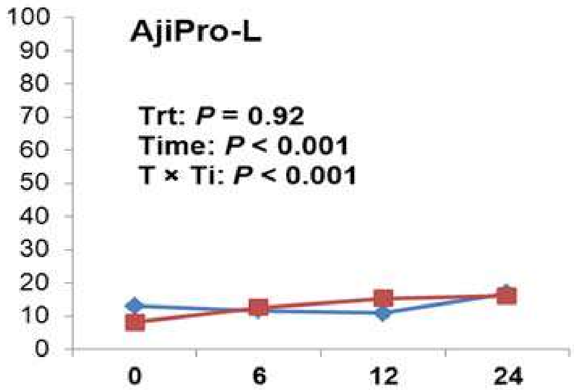
- Latin square experiments with a minimum of 7-d periods (4-d adjustment, last 3 d for blood sampling)
- RPAA supplements are mixed in the TMR 8 h before feeding

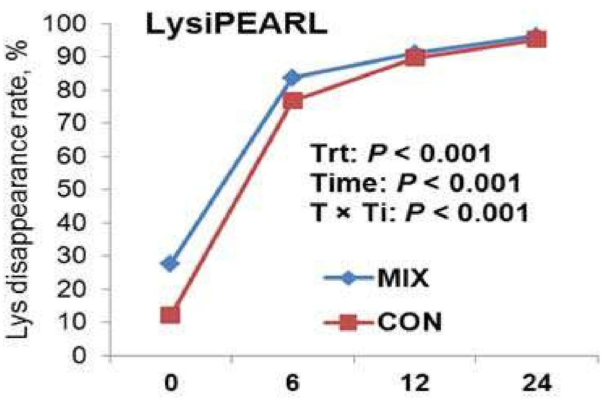

AminoShure-L
Trt: P < 0.01
Time: P < 0.001
T×Ti: P < 0.001
LD
HD
MEGAMINE-L
Trt: P = 0.13
Time: P < 0.001
T×Ti: P = 0.18
MetaboLys
Trt: P = 0.44
Time: P < 0.001
T×Ti: P = 0.02
LD
HD
AjiPro-L
Trt: P = 0.83
Time: P < 0.01
T×Ti: P = 0.39
LysiPEARL
LD
HD
Trt: P = 0.9
Time: P < 0.001
T×Ti: P = 0.64
USA Lysine
Trt: P = 0.89
Time: P < 0.001
T×Ti: P = 0.6
Lys release rate, %
0
6
18
24

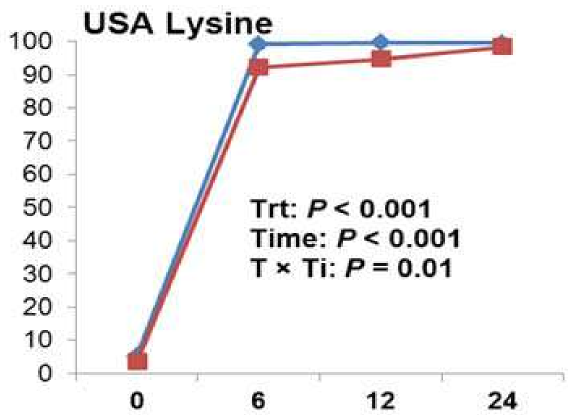

AminoShure-L
Lys disappearance rate, %
MIX
CON
Trt: P = 0.12
Time: P < 0.001
T × Ti: P = 0.62
0
6
12
24


MEGAMINE-L
Trt: P = 0.06
Time: P < 0.001
T × Ti: P = 0.04
0
6
12
24


MetaboLys
Lys disappearance rate, %
MIX
CON
Trt: P = 0.03
Time: P < 0.001
T × Ti: P = 0.02
0
6
12
24


AjiPro-L
Trt: P = 0.92
Time: P < 0.001
T × Ti: P < 0.001
0
6
12
24


LysiPEARL
Lys disappearance rate, %
Trt: P < 0.001
Time: P < 0.001
T × Ti: P < 0.001
MIX
CON
0
6
12
24


USA Lysine
Trt: P < 0.001
Time: P < 0.001
T × Ti: P = 0.01
0
6
12
24

# University of New Hampshire approach for using the plasma free AA dose-response approach

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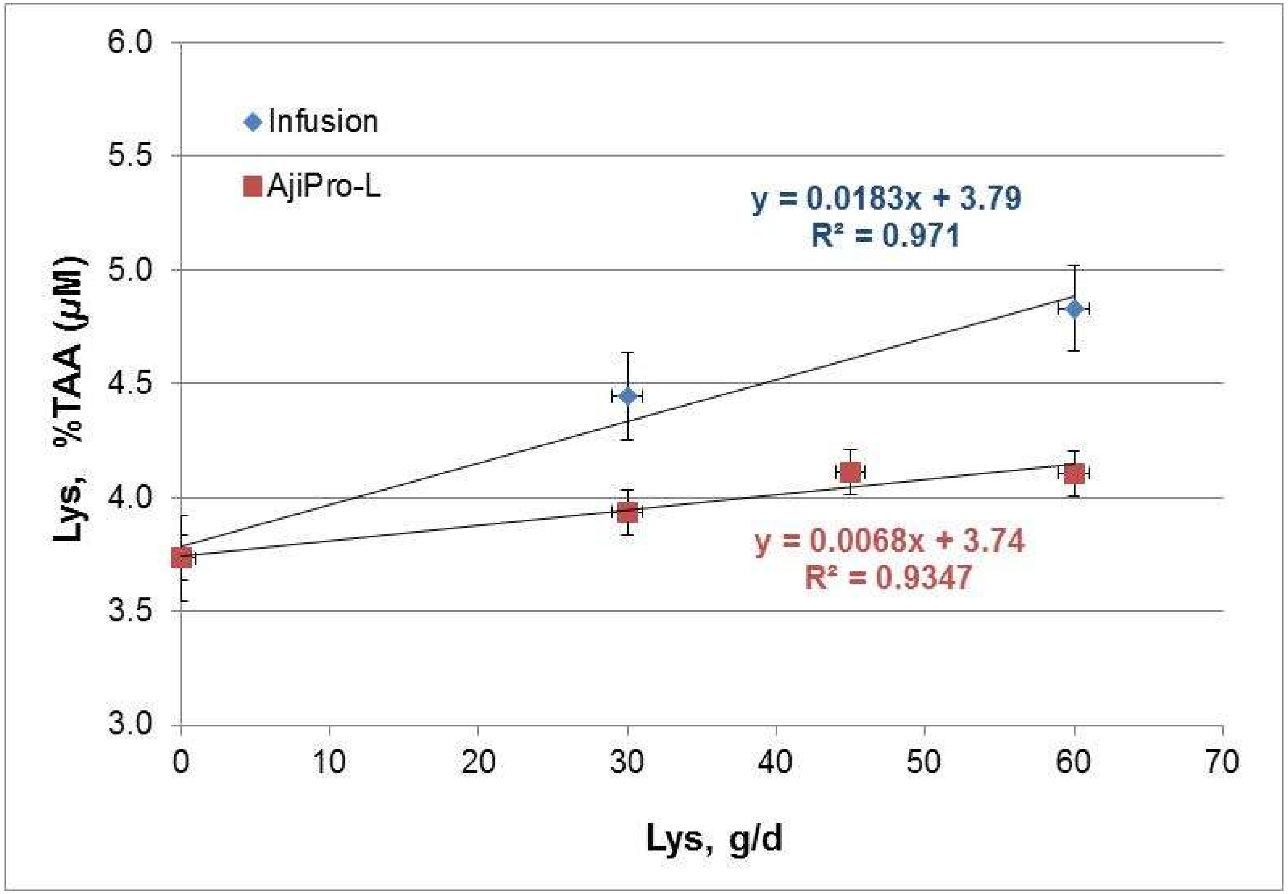
- Latin square experiments with a minimum of 7-d periods (4-d adjustment, last 3 d for blood sampling)
- RPAA supplements are mixed in the TMR 8 h before feeding
- Cows are fed at 8-h intervals and milked at 12-h intervals
- Blood samples are collected last 3 days of each period at 2, 4, 6 and 8 h after morning feeding.  Deproteinized plasma samples are pooled within day (across 4 sampling times) for each cow before AA analysis

# Evaluation of AjiPro-L 1G

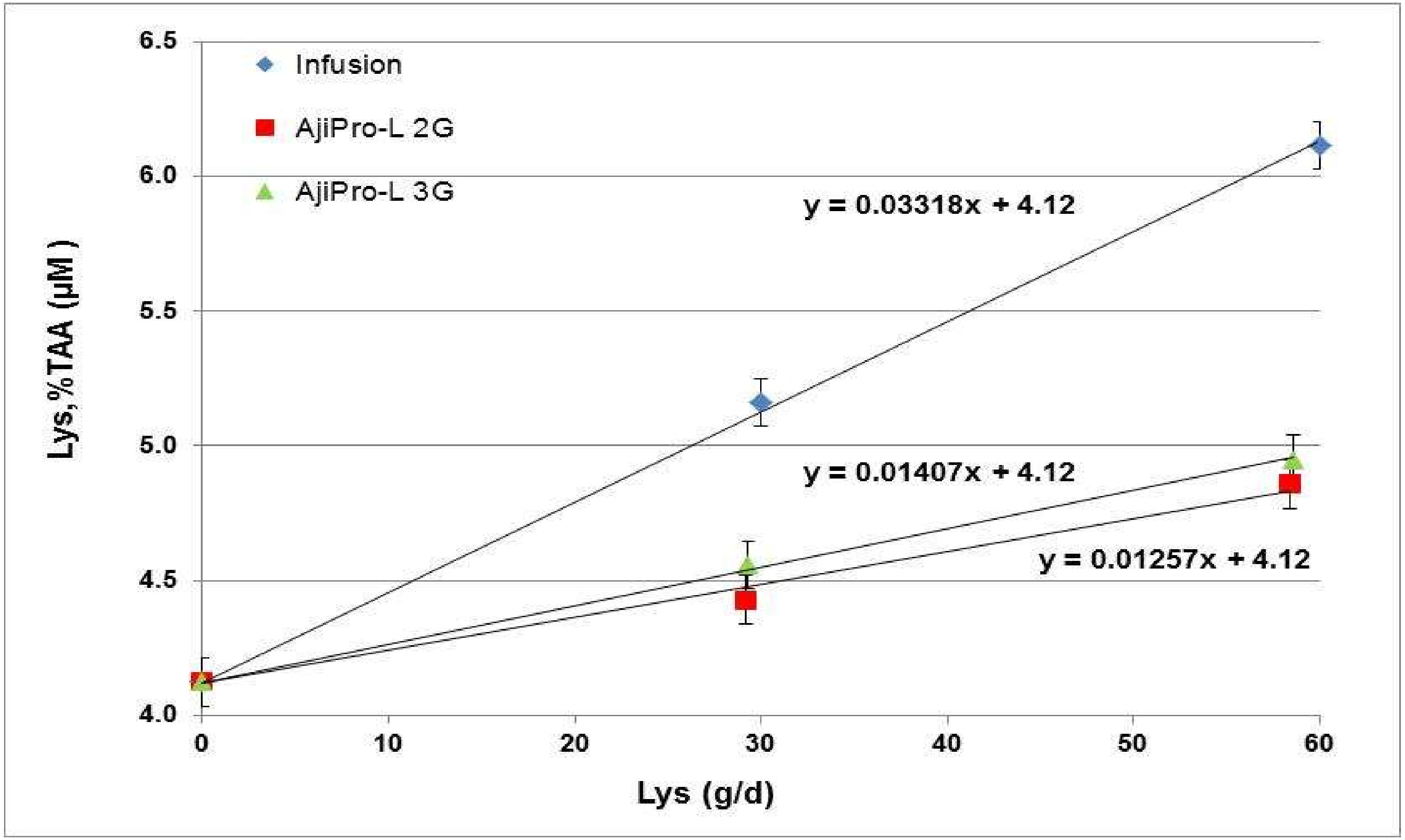



**Bioavailability:**

**(0.0068/0.0183) x 100 = 37%**

**Whitehouse et al. (2012)**

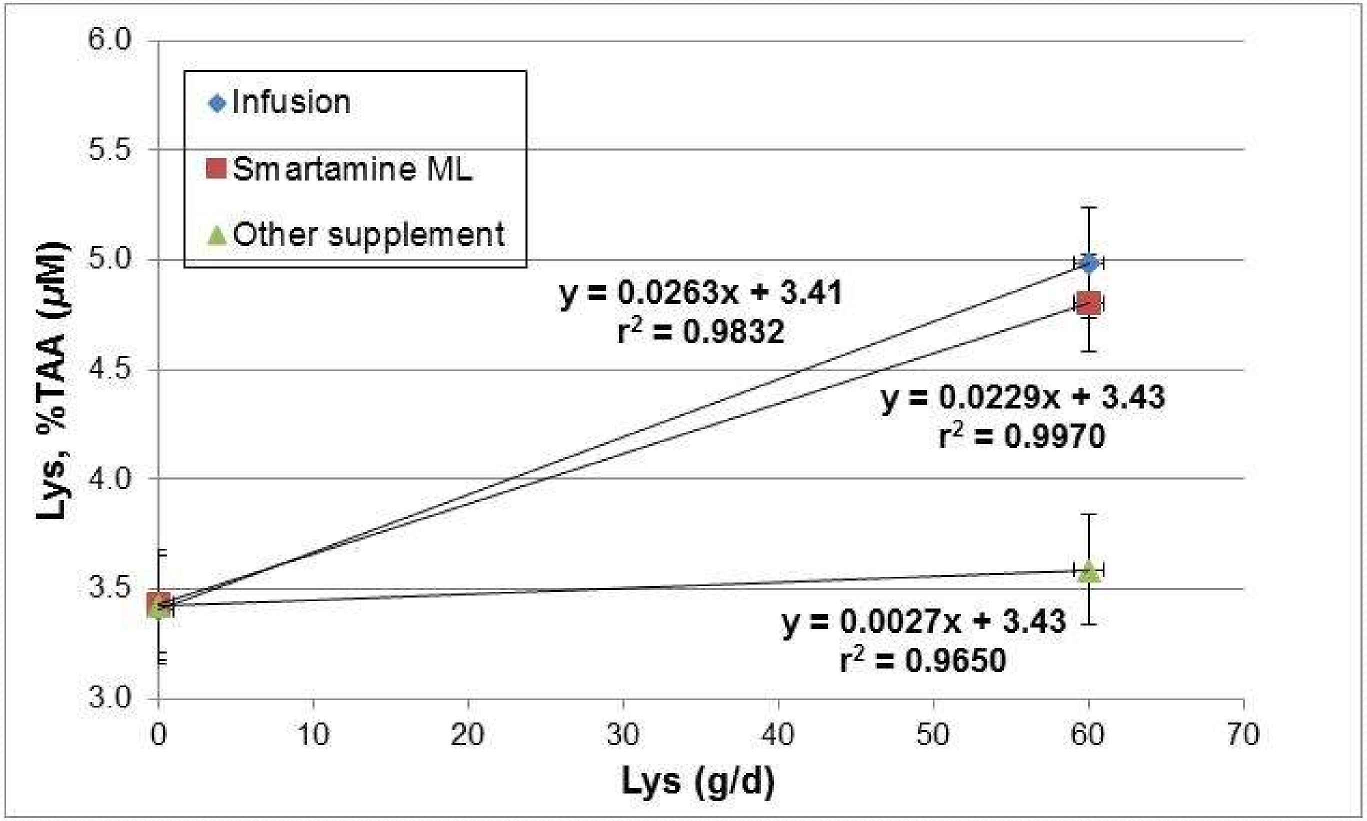
# A comparison of the relative bioavailabilities of Lys in AjiPro-L 2G and 3G




**Bioavailability values for 2G and 3G were 37.9 and 42.4%, respectively. Slopes were not different.**

**Whitehouse et al. (2017)**

# Determination of Lys bioavailability in old Smartamine ML and New AminoShure-L



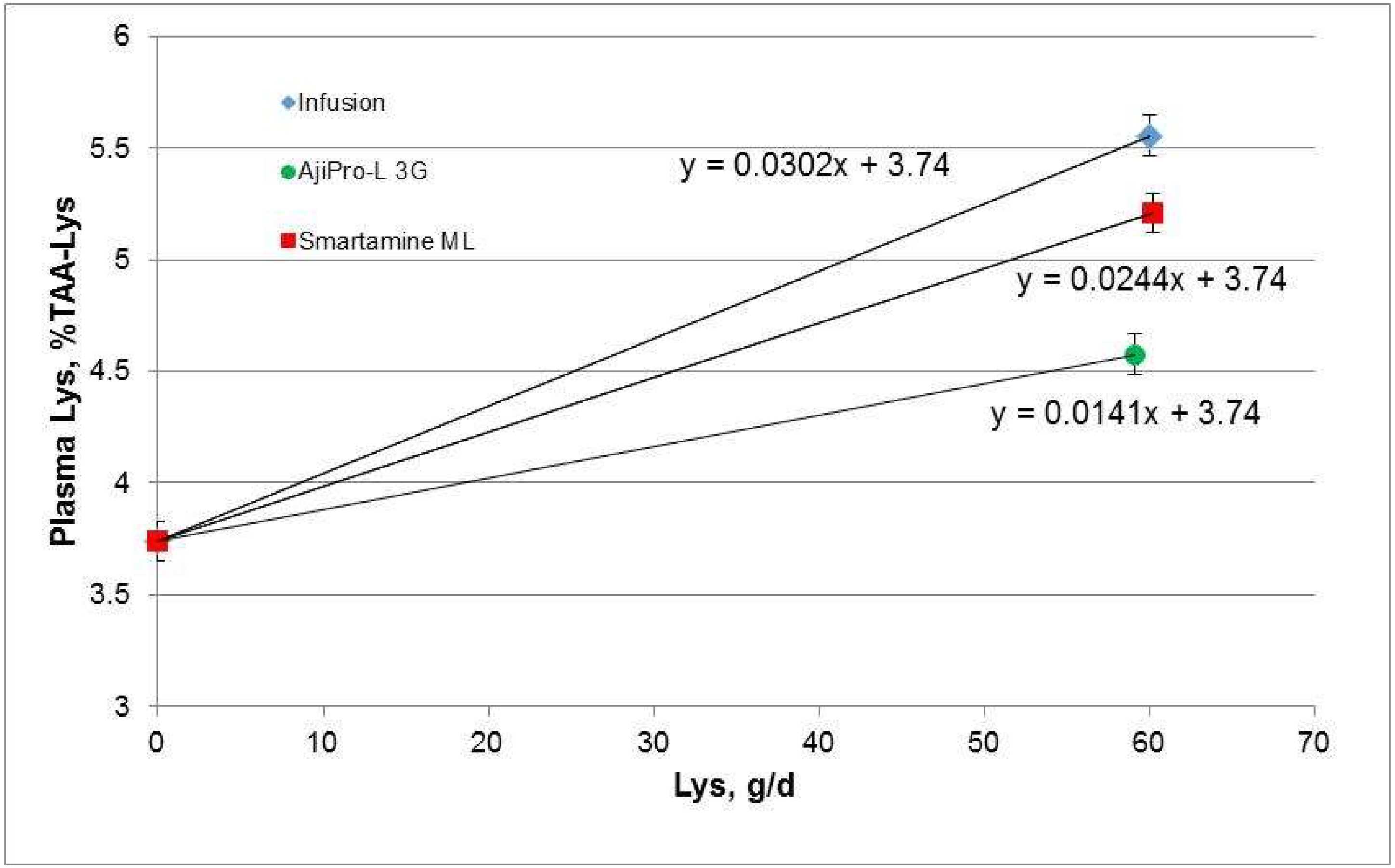

**Bioavailability:**

**Smartamine ML** (0.0229/0.0263) x 100 = <u>87.1%</u>

**New AminoShure-L** (0.0027/0.0263) x 100 = <u>10.3%</u>

**Whitehouse et al. (2017)**

# A comparison of the relative bioavailabilities of Lys in AjiPro-L 3G and Smartamine ML




**Bioavailability values for Lys in AjiPro-L 3G and Smartamine ML were 46.7 and 80.7%, respectively. CI were 41.9-51.5 and 76.1-85.3%, respectively**

**Whitehouse et al. (2018)**

**Reiners, et al. 2017. Lysine bioavailability among 2 lipid-coated lysine products after exposure to silage. Trans. Anim. Sci. 1:311-319.**

- 9 abomasally cannulated ewes (70 kg)
- Basal diet (50% corn silage, 42.8% corn, 2.5% molasses, 2.0% fishmeal, 0.66% urea) (11.2% CP)
- 9 x 9 Latin square
  - ✓ Basal
  - ✓ Infused Lys HCL (5 and 10 g)
  - ✓ Fed Lys HCL (5 and 10 g)
  - ✓ LysiPEARL (EB) (5 and 10 g)
  - ✓ USA Lysine (EC) (5 and 10 g)

**Reiners, et al. 2017. Lysine bioavailability among 2 lipid-coated lysine products after exposure to silage. Trans. Anim. Sci. 1:311-319.**

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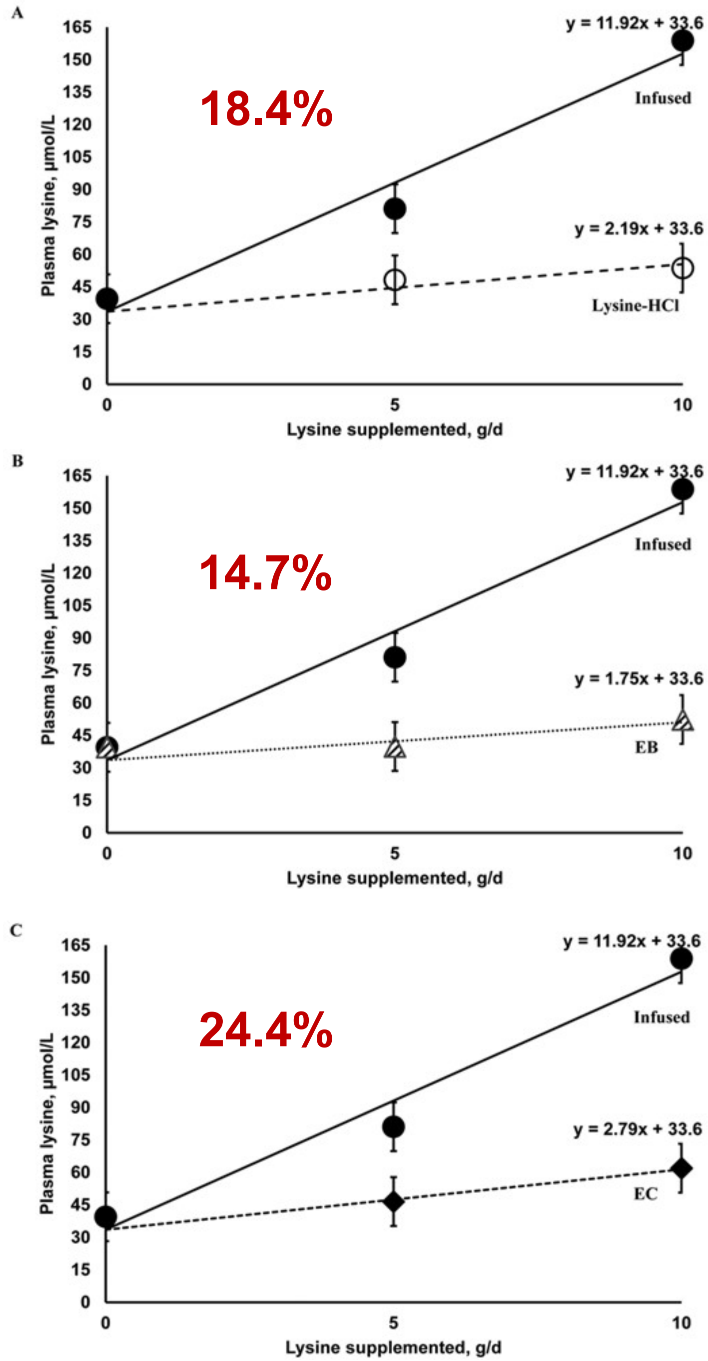
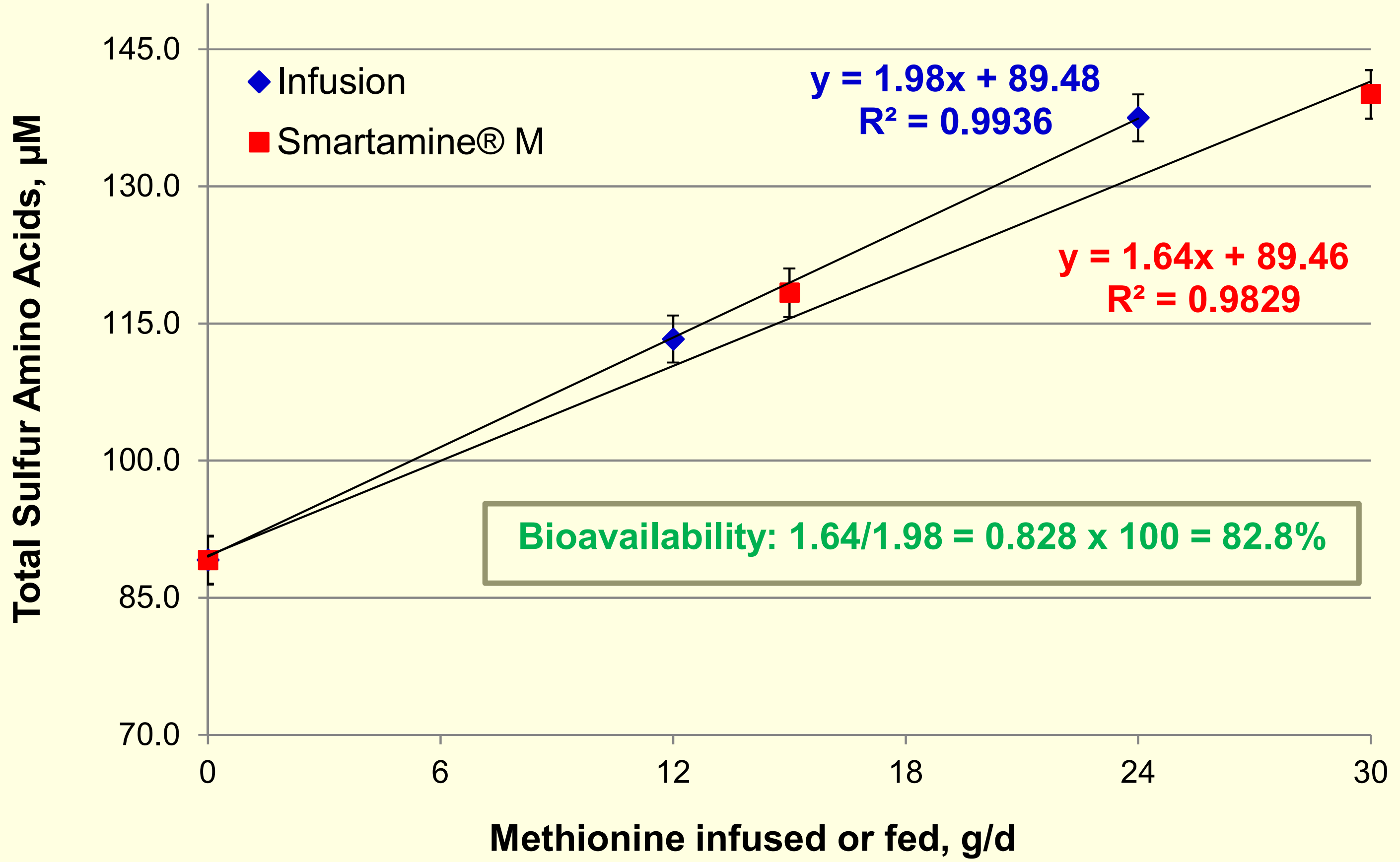


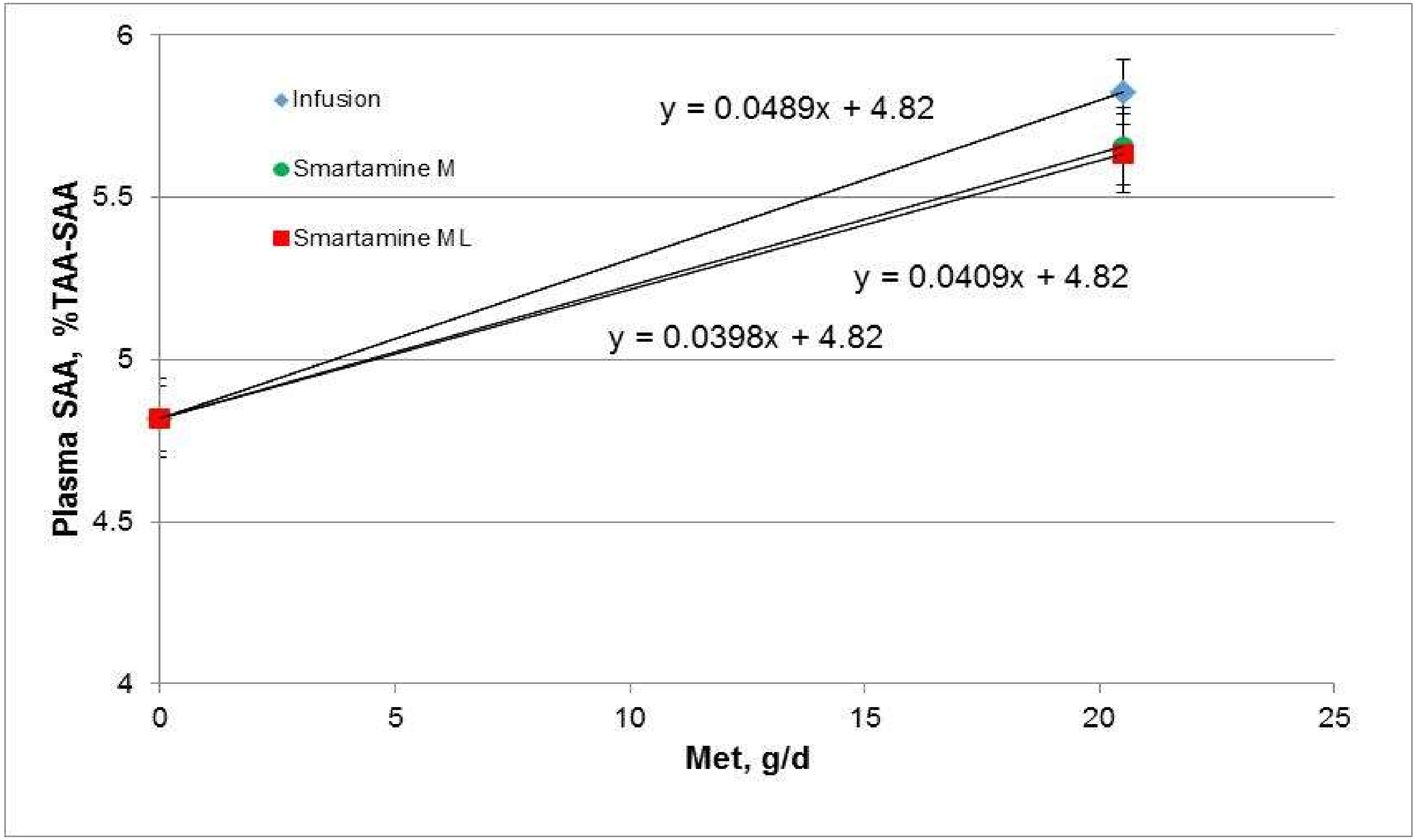
# Changes in plasma free sulfur AA concentrations with increasing amounts of infused or fed Met[1]




[1]Two 5 x 5 replicates (2013, 2014)

Chirgwin *et al.* (2015)

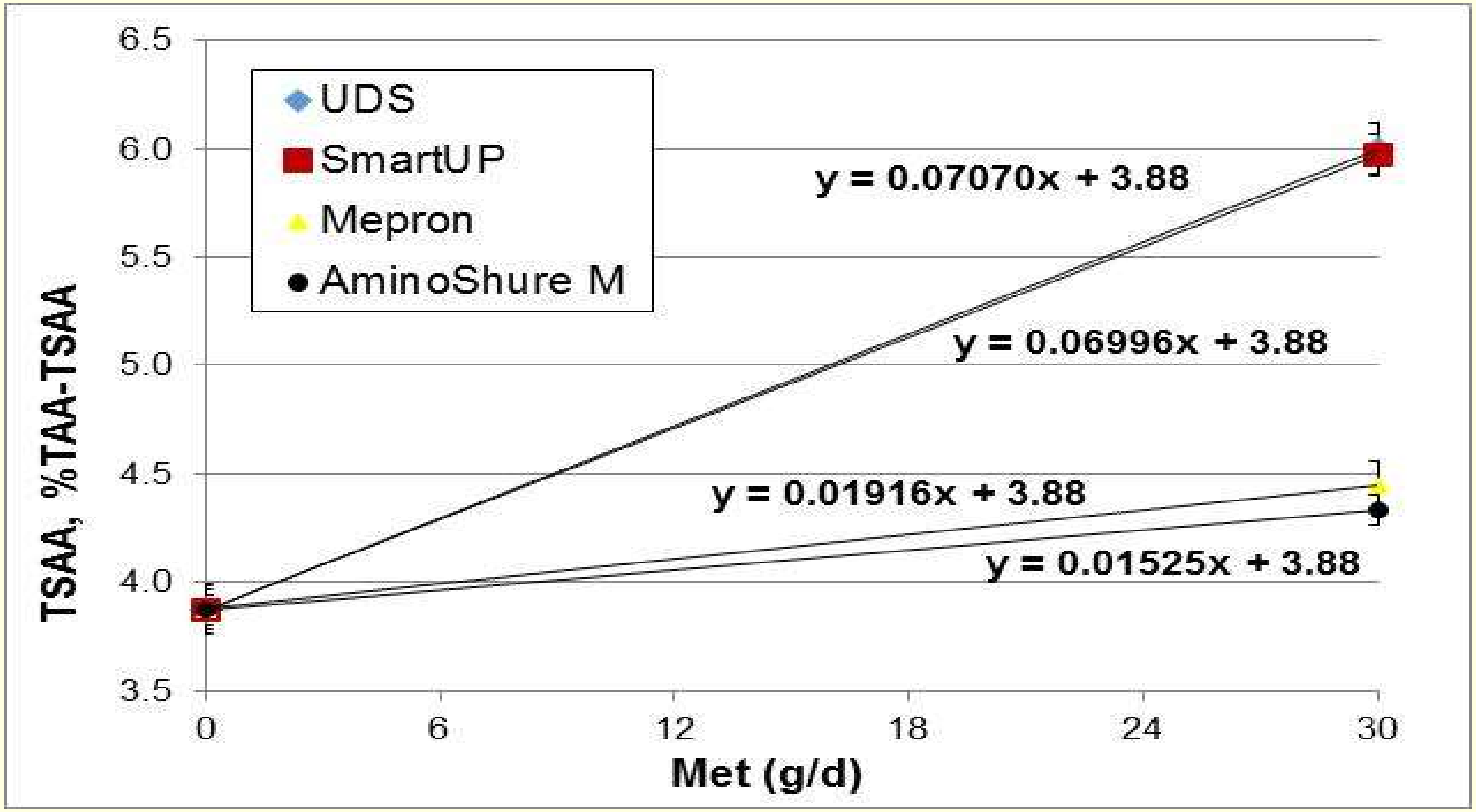
# A comparison of the relative bioavailabilities of Met in Smartamine M and Smartamine ML




**Bioavailability values for Met in Smartamine M and Smartamine ML were 83.5 and 81.3%, respectively. CI were 78.2-88.8 and 78.0-84.6%, respectively**

**Whitehouse et al. (2018)**

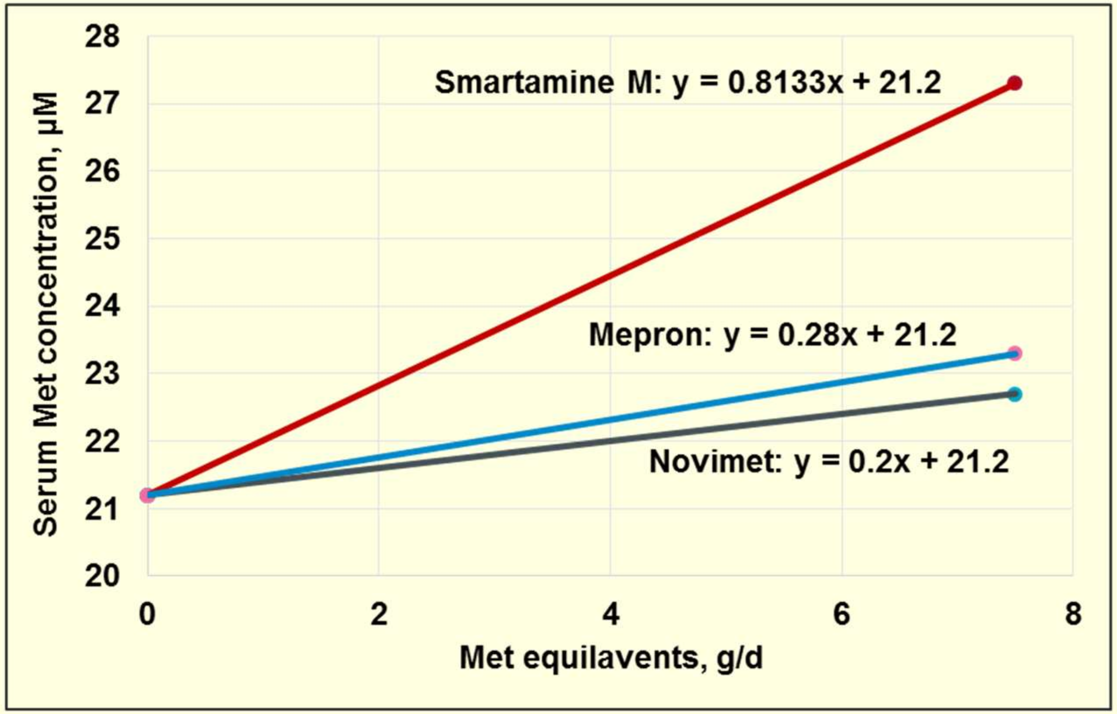
## Comparative bioavailability of four RP-Met products using the plasma free AA dose-response method using lactating cows fed a Met-deficient diet




- Two 5 x 5 Latin squares
- Compared to SmartUP, the relative Met bioavailabilities of Mepron and AminoShure M were 27 and 22 %, respectively

**Whitehouse *et al.* (2015)**

# Novimet trial




Actual Met bioavailability:

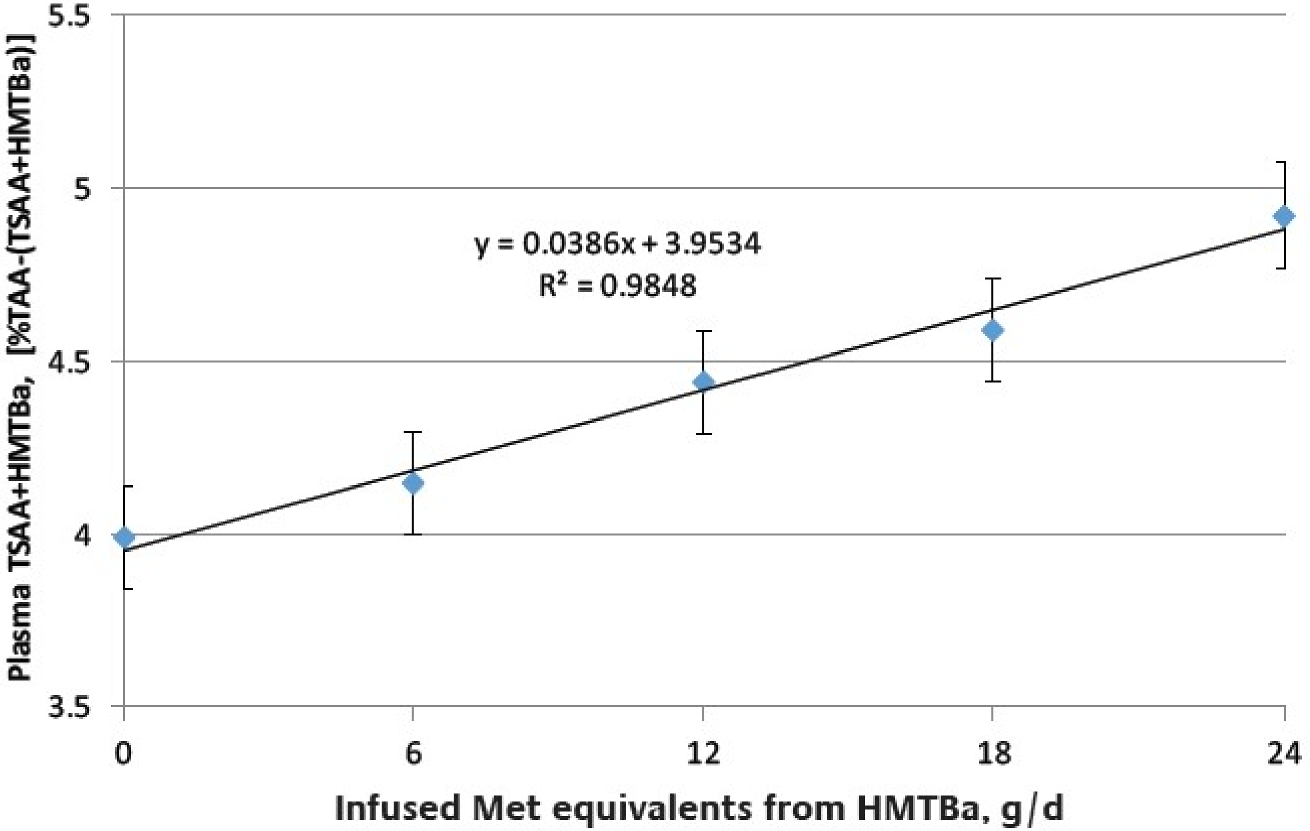
Smartamine M – 80%
Mepron – 27.5%
Novimet – 19.7%
(different from supplier provided 68%)

**g of Metabolizable Met:**

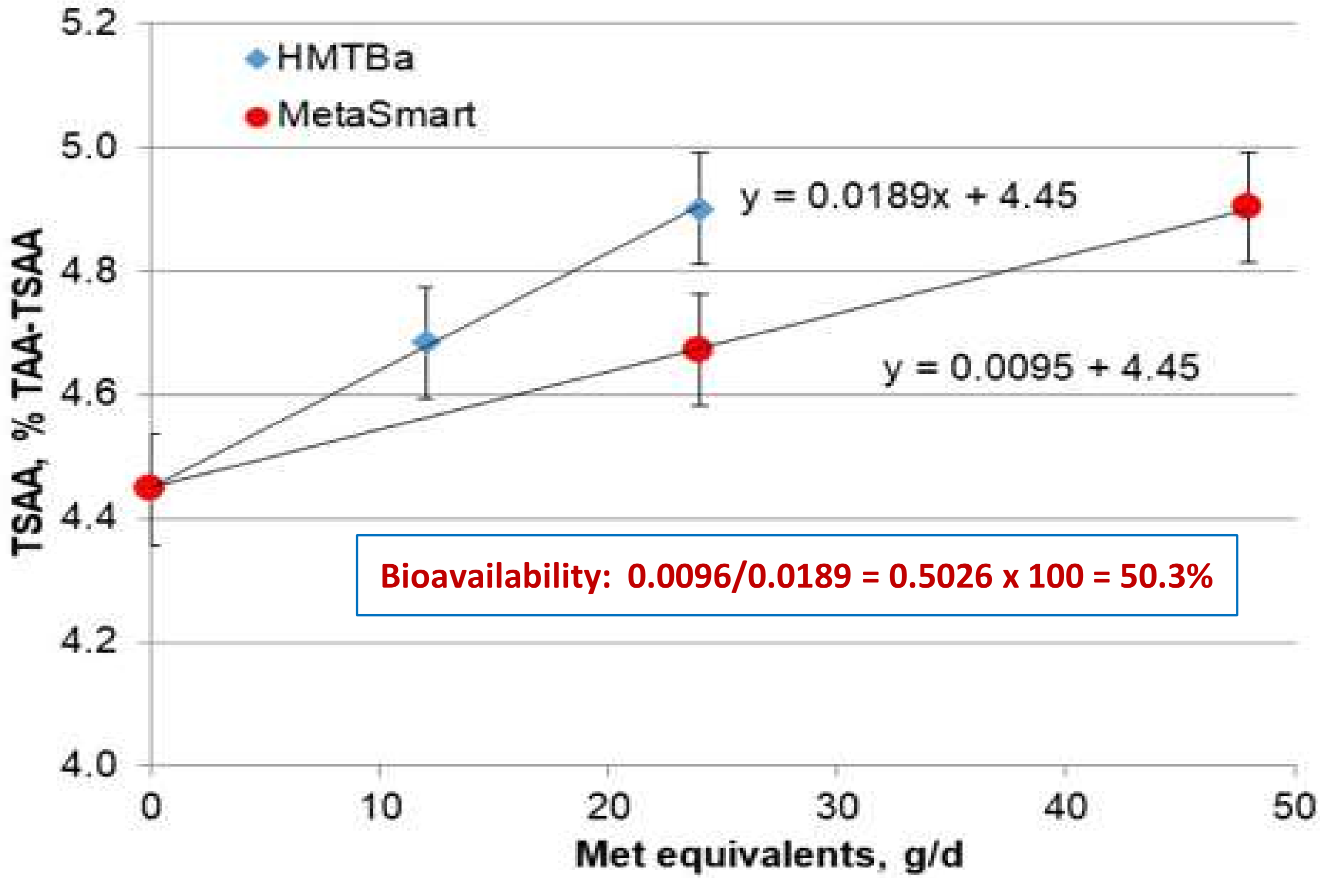
Smartamine M – 600 g/ kg of product
Mepron – 234 g/ kg of product
Novimet – 99 g/ kg of product

**Zang *et al.* (2016)**

# Changes in plasma free sulfur AA concentrations with increasing amounts of infused methionine analog (HMTBa)



# Changes in plasma free sulfur AA concentrations with increasing amounts of infused or fed MetaSmart



# Take home messages

1. **DO NOT** feed a "rumen protected AA supplement" unless you have seen confirmed estimates of "bioavailability" obtained with lactating cows using the plasma AA dose-response method
2. **CONFIRM** that the RPAA supplement was mixed and consumed with the rest of the diet during the experiment
3. **DO NOT** accept comparative milk production data as "proof of claims of AA bioavailability". Such data "always" favors inferior products

Cows respond to increased absorption of a nutrient only if that nutrient is the most limiting factor for production. Therefore, in a comparative of different sources of that nutrient, "all" cows fed the best product must remain deficient in that nutrient and no other factor can be limiting production. If that isn't the case, then the superiority of the best product won't be seen, and the inferior product(s), relative to the superior product, will look better than they are.