



# THE NEW GOLD STANDARDS IN RP-METHIONINE BIOAVAILABILITY TESTING

Today's dairy nutritionists use state-of-the-art computing software and algorithms to predict nutrient requirements, model animal performance and formulate diets with a great deal of precision. This level of exactitude is entirely dependent on accurately characterizing the nutrient content of feedstuffs to maximize animal performance and reduce ration costs.

To ensure a high degree of accuracy, nutritionists employ highly sophisticated analytical services to guickly and accurately characterize the nutrient content of feed ingredients. Once characterized, the next challenge comes in measuring nutrient bioavailability which is difficult, especially when it comes to amino acids in dairy cattle. This is due to the complicating influence of the rumen. In the area of amino acid bioavailability, researchers at the Ohio State University and Virginia Tech have independently developed two relatively new testing methodologies. These protocols provide a higher level of accuracy and deliver universal efficacy, regardless of product formulation.

## The Old Standard

**Plasma Amino Acid Dose Response Method** – This protocol has been used for many years and was developed specifically to measure the "area under the curve." Historically this method has favored products that release their methionine payload very quickly into the gut while underestimating the bioavailability of products that release their payload more slowly. It has been speculated that products with a rapid payload release will flood the blood plasma with a large spike of methionine, exaggerating the area under the curve.

Though the method has undergone several modifications to adjust for inherent variation, the test is still biased against products that meter their payload more gradually into the gut, consistently underestimating their bioavailability.

## The Gold Standard(s)

Two ground-breaking methionine testing methodologies were recently developed that can precisely measure the bioavailability of all rumen- protected methionine products, regardless of the speed of release.

**Seleno-Methionine Technique** – a noninvasive, *in vivo* methodology that utilizes selenium labeled methionine as a tracer of methionine in milk. Change in milk methionine relative to milk selenium is used to determine how much of a RP-Met product by-passes the rumen, is absorbed in the small intestine and is then utilized in the mammary gland. This technique has been proven and published as an accurate method in determining relative supply of metabolizable methionine.

**Stable Isotope Technique** – employs a jugular infusion of isotopically labeled amino acids that act as tracers in an *in vivo* system. Isotopic enrichment of plasma amino acids in response to the jugular infusion is measured and modeled in a 4-pool model (*which encompasses protein turnover pools*) to derive amino acid plasma entry rates in animals fed diets containing an ingredient of interest. This is a noninvasive, *in vivo* methodology that has been proven to provide accurate and precise assessments of amino acid absorption for feed ingredients in a more typical feeding environment (*i.e. no need for ruminally or intestinally cannulated animals, abomasal infusions, pulse doses, etc.*).

## **Comparing Results**

Figure 1 demonstrates the differences between the new and old methodologies. The results using the Stable Isotope and Seleno-Methionine methods matched the manufacturer's claim, while the Plasma AA Pulse Response method grossly underestimated the product bioavailability.

## **FIGURE 1**

AminoShure<sup>™</sup>-XM bioavailability test results using different testing methods

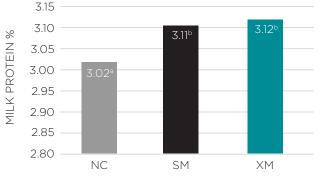
| Testing Methodology      | Test Results |                                       |
|--------------------------|--------------|---------------------------------------|
| Stable Isotope           | 55.00%       | Manufacturer's<br>Claim<br><b>55%</b> |
| Seleno-Methionine        | 54.25%       |                                       |
| Plasma AA Pulse Response | 28.00%       |                                       |

### The Cow is the Ultimate Judge

Clinical findings should not stand on their own. Production studies are necessary to ensure the results show up in the bulk tank. Production studies with AminoShure<sup>™</sup>-XM were performed at Virginia Tech and the University of Delaware verifying the results seen in the Stable Isotope and Seleno-Methionine bioavailability studies. As shown in Figures 2 and 3, cows supplemented with AminoShure-XM produced more milk protein than controls and, when fed at a similar level to Smartamine, exhibited the same response. The production studies also confirm that the results seen in the Plasma AA Pulse Response trial were artificially low and inaccurate.

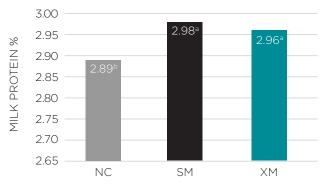
#### FIGURE 2

Effects of Supplemental RP-Methionine on Milk Protein Percent in Lactating Dairy Cows at Virginia Tech.



#### FIGURE 3

Effects of Supplemental RP-Methionine on Milk Protein Percent in Lactating Dairy Cows at University of Delaware.



NC=Negative control diet

**SM**=NC supplemented with Smartamine® M

XM=NC supplemented with AminoShure™-XM a,b Means with different superscript letters within the same row are significantly different (P < 0.05).

## **Growing Together**

Modern technology is developing at an unprecedented pace and Balchem is committed to matching strides by leveraging the expertise of our people, staying grounded in science, and delivering the results our customers need for a sustainable future.

# References:

Ardalan, M., Vargas-Rodriguez, G.I. Zanton, M. Vazquez-Anon, E. Titgemeyer, and B. Bradford. 2016. Effectiveness of two ruminally protected methionine sources for lactating dairy cows. Kansas Agricultural Experiment Station Research Reports: Vol. 2: Iss. 9.

Barnard, A.M., M.K. Conklin, K. Estes, B.A. Barton, C. Zimmerman, and T.F. Gressley. 2018. Assessing three levels of a rumen-protected methionine prototype on dairy cow performance. J. Dairy Sci. Suppl. 2, 101:90.

Fleming, A.J., K. A. Estes, H. Choi, B. A. Barton, C. A. Zimmerman, and M. D. Hanigan. 2019. Assessing bioavailability of ruminally protected methionine and lysine prototypes. J. Dairy Sci. 102:4014-4024.

Graulet, B., C. Richard, and J.C. Robert. 2005. Methionine availability in plasma of dairy cows supplemented with methionine hydroxy analog isopropyl ester. J. Dairy Sci. 88:3640-3649.

Huang, X., K.A. Estes, P.S. Yoder, C. Wang, N. Jiang, T. Pilonero, and M.D. Hanigan. 2019. Assessing availability of amino acids from various feedstuffs in dairy cattle using a stable isotope-based approach. J. Dairy Sci. 102:10983-10996. Lapierre, H., G. Holtrop, A.G. Calder, J. Renaud, and G.E. Lobley. 2012. Is D-methionine bioavailable to the dairy cow? J. Dairy Sci. 95;353-362.

Rulquin, H. and J. Kowalczyk. 2003. Development of a method for measuring lysine and methionine bioavailability in rumen-protected products for cattle. J. Animal and Feed Sci. 12:465-474.

Sudekum, K.H., S. Wolffram, P. Ader, and J.C. Robert. 2004. Bioavailability of three ruminally protected methionine sources in cattle. Anim. Feed Sci. and Tech. 113:17-25.

Weiss, W.P. and N.R. St-Pierre. 2009. A method to quantify changes in supply of metabolizable methionine to dairy cows using concentrations of selenium in milk. J. Dairy Sci. 92:2835-2842.

Weiss, W.P., A.W. Tebbe, K. Estes, and C. Zimmerman. 2019. Metabolizable methionine content of rumen protected.



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