

## **Effectiveness of Two Ruminally Protected Methionine Sources for Lactating Dairy Cows**

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### **Summary**

Two sources of ruminally protected methionine were tested for their ability to provide available methionine to lactating dairy cattle. Based on milk protein yield and milk protein percent, NTP-1401 (an unreleased product from Novus International, Inc., St. Charles, MO) and Smartamine (Adisseo, Alpharetta, GA) provided similar amounts of available methionine to the cows. These two products led to different methionine-related compounds appearing in blood plasma, suggesting that they contained different methionine precursors.

Key words: availability, ruminally protected methionine

### **Introduction**

Feeding diets with excessively high crude protein concentrations to meet dairy cow requirements for metabolizable protein leads to excretion of excess nitrogen in manure. One approach to reducing dietary crude protein content is supplementing specific limiting amino acids to the cow, but these supplemental amino acids need to be protected from ruminal degradation to be effective nutrient sources for cattle. To effectively use these ruminally protected amino acids in diet formulation, it is critical to know the extent to which the amino acids are available to the cow, that is, they must be protected from ruminal degradation, yet available for intestinal digestion.

In the past several decades there has been a great deal of interest in investigating the role of methionine on the yield of milk components in lactating cows. Methionine is typically the most limiting amino acid for milk protein synthesis and optimal dairy production. As a consequence of the beneficial effects of methionine supplementation, such as increased milk protein content and yield, various ruminally protected methionine products are commercially available.

The objective of this experiment was to evaluate the relative effectiveness of two different ruminally protected methionine sources for supporting milk production in dairy cows. One of the sources is a product that has been extensively evaluated (Smartamine),

<sup>1</sup> Novus International, Inc., St. Charles, MO.

whereas the other is a newly developed product that is not yet on the market (as of November 2016).

## Experimental Procedures

All procedures involving animals were approved by the Kansas State University Institutional Animal Care and Use Committee.

Twenty-one Holstein dairy cows between 80 and 140 days in milk (11 primiparous and 10 multiparous) were housed at the Kansas State University Dairy Teaching and Research Center in tie-stalls with rubber mats and wood shavings. Cows were used in 4 replicated  $5 \times 5$  Latin squares with treatment sequences balanced for carryover effects as best possible; 2 squares contained only primiparous cows and 2 squares contained multiparous cows. The extra animal was provided treatments in a sequence that was identical to another primiparous cow. Treatments were: a) control (no methionine supplement); b) NTP-1401, a methionine-providing product from Novus International Inc., supplemented at 7.5 grams of product daily; c) NTP-1401 supplemented at 15 grams of product daily; d) Smartamine-M (Adisseo, Alpharetta, GA), a ruminally protected methionine product, supplemented at 7.5 grams of product daily; and e) Smartamine-M supplemented at 15 grams of product daily. Smartamine is methionine coated with poly (2-vinylpyridine-co-styrene) sensitive to acidic pH in the abomasum, and contains 75% DL-methionine. The diet (Table 1) was fed as a total mixed ration once daily and added to bunks twice daily. Cows were individually fed at 6:00 a.m. and 4:00 p.m. for ad libitum intake with free access to water. Treatments were top-dressed on the total mixed ration and hand mixed with top third of diet at the time of feeding. Total daily feed was adjusted to allow for approximately 10% refusals. The diet was evaluated by the Cornell Net Carbohydrate and Protein System (version 4.0) and found to meet the metabolizable protein and energy requirements when DMI was 25.58 kg/d for a lactating Holstein cow producing 45 kg/d of milk with 3.5% milk fat and 3.00% milk true protein. Diets were formulated to have a moderate level of crude protein (16%), a predicted deficiency of metabolizable methionine (1.85% of metabolizable protein), and a sufficient provision of lysine (6.8% of metabolizable protein). Experimental periods were each 14 days long and included 10 days for adaptation to treatments and 4 days for sample and data collection.

Cows were milked 3 times daily in a milking parlor at 7:00 a.m., 3:00 p.m., and 11:00 p.m. Milk yields were recorded at each milking. Feed intake and milk production were measured over the final 4 days of each period. Milk samples were analyzed at the Heart of America DHIA, Manhattan, KS. Blood samples for plasma amino acid analysis were collected from the tail vein from each cow at 4 hours after the afternoon feeding on the final day of each period.

## Results and Discussion

Diets were formulated to be deficient in metabolizable methionine, but to provide sufficient lysine. Because our diets were deficient in methionine, we expected increases in milk protein percentage and yield if the products were effective in providing bioavailable methionine to the cows. Previous work has shown that milk protein is a sensitive indicator of methionine supply and a useful response for determining the effectiveness

of ruminally protected methionine sources. In our study, milk protein was the primary response criteria.

Dry matter intake and milk production and composition are shown in Table 2. Dry matter intake was not affected by methionine supplementation ( $P \geq 0.14$ ). Although supplementation with methionine can sometimes increase milk production in dairy cows, milk yield in our study averaged 46.2 kg/day and was not affected by the methionine supplements. Similarly, milk fat yield and percentage (3.49%) were not affected by methionine supplementation.

Milk protein percentage and milk protein yield increased linearly with methionine supplementation ( $P < 0.01$ ), indicating that methionine increased protein synthesis in the mammary gland. There were no differences between the two methionine sources for milk protein percentage or yield, suggesting that they provided similar amounts of available methionine to the cows. Linear regressions of milk protein yield (Figure 1) and milk protein percentage (Figure 2) against supplement amount within source led to slope ratios (NTP-1401/Smartamine) of 95% for protein percentage (not different from 100%,  $P = 0.65$ ) and 84% for protein yield (not different from 100%,  $P = 0.60$ ), further suggesting no differences between sources for increasing milk protein.

In addition to measuring production responses of the cows, we also measured plasma amino acid concentrations to further characterize the effectiveness of the products. For protein synthesis, cattle require that the natural isomer of methionine (L-methionine) be available within the cell. However, methionine supplements are typically synthesized chemically, and these products therefore contain other forms of methionine that the cow subsequently must convert to L-methionine within the body. Smartamine-M contains DL-methionine, which is a 50:50 mixture of the natural L-methionine and the unnatural D-methionine. It has been previously demonstrated that cattle are able to convert D-methionine to L-methionine, and thus D-methionine can be an available source of methionine for cattle. Methionine hydroxy analog (MHA; 2-hydroxy-4-methylthio-butyric acid) is also a synthetic methionine source. Although MHA is not a true amino acid, it is metabolized by the cow to produce L-methionine after it is absorbed from the gut.

The effects of NTP-1401 and Smartamine on plasma concentrations of L-methionine, D-methionine, the hydroxy analog of methionine (MHA; 2-hydroxy-4-methylthio-butyric acid), and total methionine equivalents (the sum of L-methionine, D-methionine, and MHA) are shown in Figures 3 through 6. Plasma L-methionine (the natural form of methionine) was linearly increased ( $P < 0.01$ ) when either Smartamine or NTP-1401 was included in the diet, and there were no differences between the two methionine sources ( $P = 0.61$ ). Supplementation with Smartamine significantly increased plasma D-methionine, whereas NTP-1401 did not increase plasma D-methionine. Because D-methionine is an unnatural isomer of methionine, plasma concentrations were below detection limits for cows fed the control diet. Smartamine contains D-methionine, so its ability to increase plasma D-methionine is not surprising. The inability of NTP-1401 to increase plasma D-methionine suggests that this product did not contain D-methionine. Cows receiving NTP-1401 demonstrated increases in plasma MHA, whereas those fed Smartamine did not show any increase. The lack of response for

Smartamine was expected because Smartamine does not contain MHA. The increase in plasma MHA in response to NTP-1401 supplementation suggests that the NTP-1401 contains MHA that is protected from ruminal degradation.

Because the two methionine products provided different methionine precursors to the cows, we added the concentrations of the different methionine-related compounds to derive an estimate of total availability based on the blood data. Plasma concentrations of total methionine equivalents increased linearly when either methionine source was supplemented, and there were no differences between the two sources (Figure 6). This would be suggestive of similar bioavailable methionine provision by the two products, which agrees with the observations for milk protein concentration and amount.

In summary, we compared two ruminally protected methionine sources for lactating cows. Smartamine, a product extensively used in the dairy industry, and NTP-1401, a new product that is not yet commercially available, led to similar increases in milk protein as well as in concentrations of total methionine equivalents in plasma, suggesting that the products provided similar amounts of available methionine to the cows.

**Table 1. Ingredient composition of the diet**

Ingredient	% of DM
Corn silage	29.9
Alfalfa	23.9
Whole cottonseed	3.9
Finely rolled corn	20.1
Solvent soybean meal	3.4
SoyBest <sup>1</sup>	1.8
Soybean hulls	8.1
Dried molasses	2.6
Blood meal	1.5
Energy Booster 100 <sup>2</sup>	2.1
Limestone	0.47
Dicalcium phosphate	0.55
Salt	0.39
Trace-mineralized salt <sup>3</sup>	0.18
Sodium bicarbonate	0.59
Magnesium oxide	0.20
Copper sulfate	0.0053
Zinc oxide	0.0089
Selenium premix, 600 mg Se/kg	0.030
Vitamin A premix, 30,000 IU/g	0.018
Vitamin D premix, 20,000 IU/g	0.0053
Vitamin E premix, 44 IU/g	0.18
Ethylenediamine dihydriodide, 4.4%	0.0008
Rumensin 90 <sup>4</sup>	0.0075
Yeast <sup>5</sup>	0.055
Biotin premix, 220 mg biotin/kg	0.11

<sup>1</sup> Mechanically extracted soybean meal with soy lecithins added during manufacture (Grain States Soya, West Point, NE).

<sup>2</sup> Rumen bypass fat with 98% fatty acids (Milk Specialties, Eden Prairie, MN).

<sup>3</sup> Composition: > 95.5% NaCl, 0.24% Mn, 0.24% Fe, 0.05% Mg, 0.032% Cu, 0.032% Zn, 0.007% I, and 0.004% Co.

<sup>4</sup> Provided 15 mg monensin/kg diet DM (Elanco Animal Health, Indianapolis, IN).

<sup>5</sup> Diamond V XPC Yeast (Diamond V Mills, Inc., Cedar Rapids, IA).

**Table 2. Effect of supplemental methionine from the NTP-1401 or from Smartamine on milk production**

Item	Dietary treatment					SEM	<i>P</i> - value <sup>1</sup>			
	Control	NTP-1401 (g/d)		Smartamine (g/d)			Effect of methionine		Source	Source × level
		7.5	15	7.5	15		Linear	Quadratic		
No. of observations	20	21	21	21	21					
DM intake, kg/day	27.2	27.0	26.8	27.0	27.2	0.43	0.59	0.74	0.54	0.39
Milk yield, kg/day	46.4	46.4	46.0	46.1	46.4	1.1	0.72	0.89	0.99	0.29
Milk:DM intake	1.71	1.72	1.72	1.71	1.71	0.029	0.67	0.80	0.38	0.82
Protein, %	2.77	2.82	2.86	2.81	2.87	0.041	<0.001	0.94	0.99	0.10
Protein yield, kg/day	1.28	1.30	1.31	1.29	1.33	0.026	0.004	0.84	0.92	0.14
Fat, %	3.46	3.48	3.51	3.50	3.50	0.094	0.35	0.84	0.94	0.71
Fat yield, kg/day	1.59	1.60	1.61	1.61	1.61	0.041	0.53	0.99	0.77	0.99

<sup>1</sup> Probability that effects are due to random chance; values smaller than 0.05 are considered significant. Effects of methionine are for averages across both methionine sources. Source is a comparison of NTP-1401 to Smartamine averaged across both levels. Source × level determines if the effects of the two methionine sources are similar across both levels of supplementation.

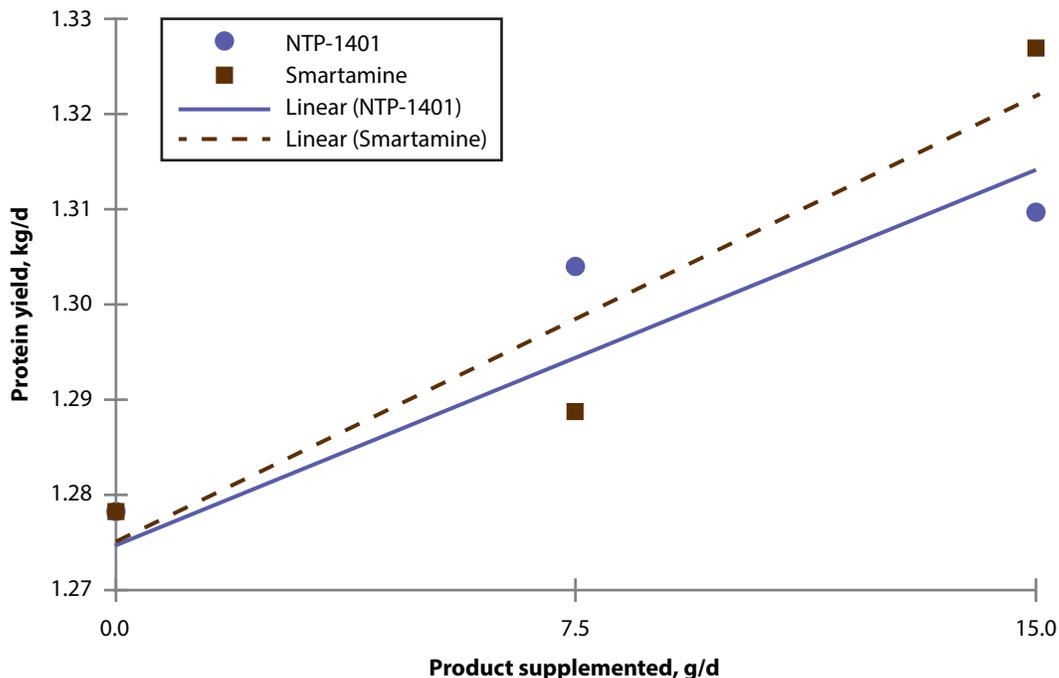


Figure 1. Linear regression of milk protein yield against supplemental methionine from NTP-1401 or Smartamine. Milk protein yield =  $1.275 + 0.0026 (\pm 0.0010) \times \text{NTP-1401} + 0.0031 (\pm 0.0010) \times \text{Smartamine}$ . The slopes of the different methionine sources were not significantly different ( $P = 0.60$ ).

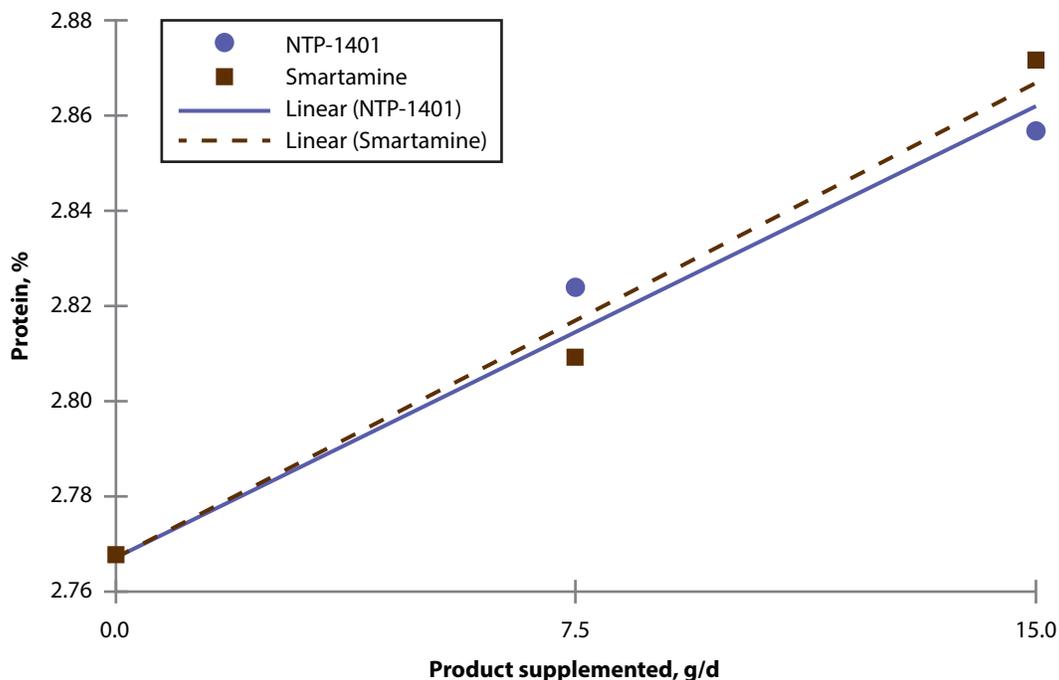


Figure 2. Linear regression of milk protein percentage against supplemental methionine from NTP-1401 or Smartamine. Milk protein percentage =  $2.77 + 0.0064 (\pm 0.0008) \times \text{NTP-1401} + 0.0067 (\pm 0.0008) \times \text{Smartamine}$ . The slopes of the different methionine sources were not significantly different ( $P = 0.65$ ).

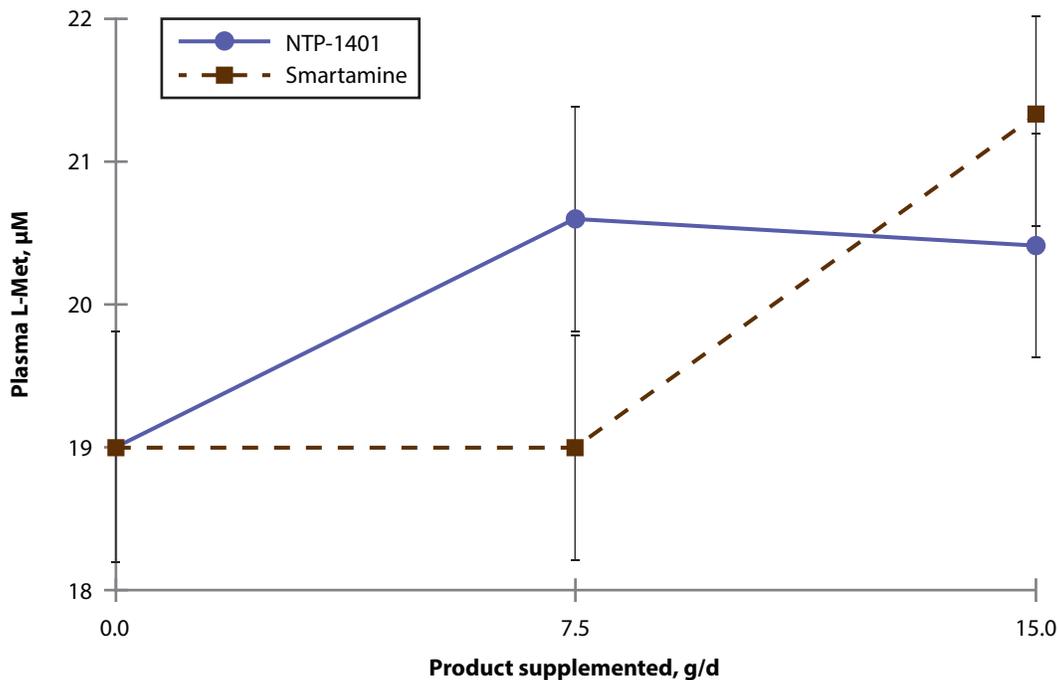


Figure 3. Effects of supplemental NTP-1401 and Smartamine on plasma concentrations of L-methionine. Linear effect of methionine supplementation ( $P = 0.04$ ); quadratic effect of methionine supplementation ( $P = 0.84$ ); differences between sources ( $P = 0.61$ ); and source  $\times$  level interaction ( $P = 0.08$ ).

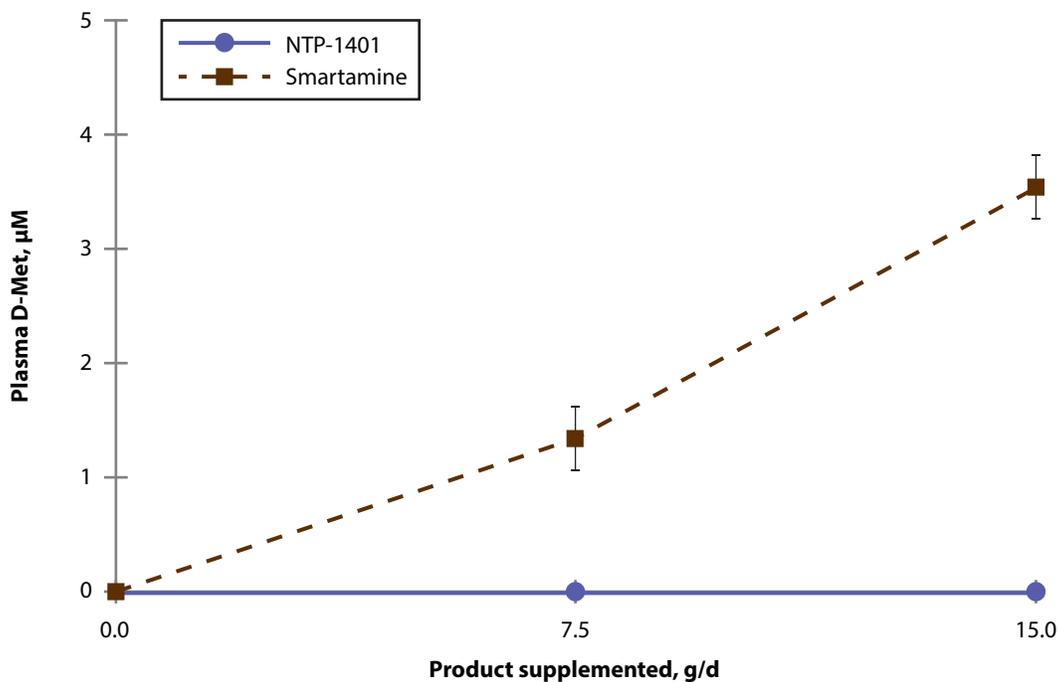


Figure 4. Effects of supplemental NTP-1401 and Smartamine on plasma concentrations of D-methionine. Linear effect of methionine supplementation ( $P < 0.0001$ ); quadratic effect of methionine supplementation ( $P = 0.44$ ); differences between sources ( $P < 0.0001$ ); and source  $\times$  level interaction ( $P = 0.0001$ ).

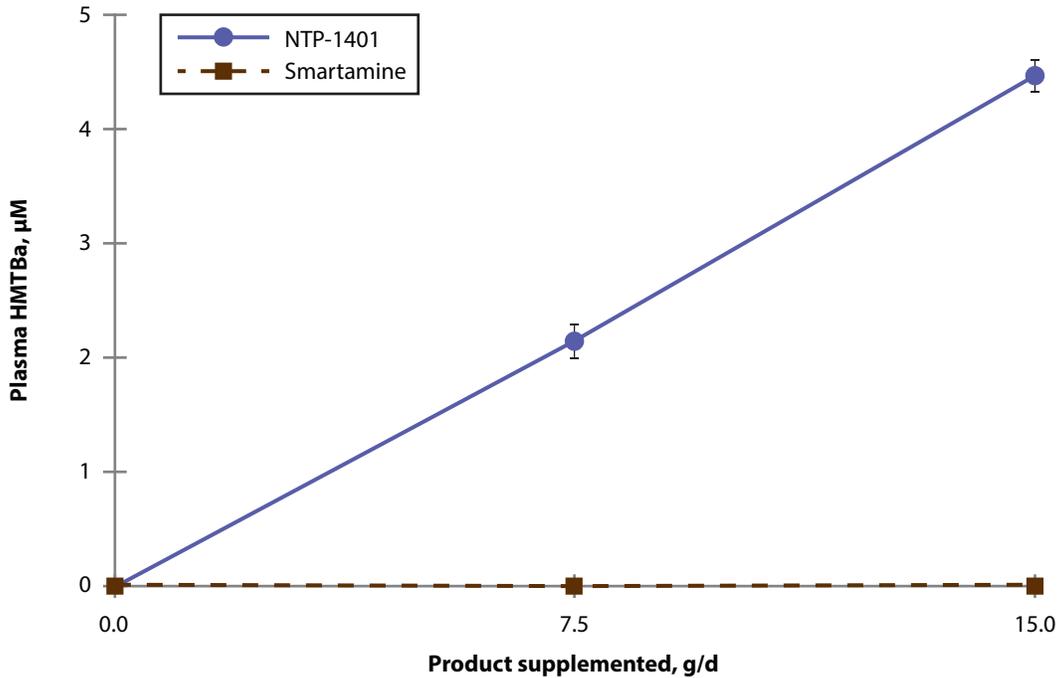


Figure 5. Effects of supplemental NTP-1401 and Smartamine on plasma concentrations of methionine hydroxy analog. Linear effect of methionine supplementation ( $P < 0.0001$ ); quadratic effect of methionine supplementation ( $P = 0.44$ ); differences between sources ( $P < 0.0001$ ); and source  $\times$  level interaction ( $P < 0.0001$ ).

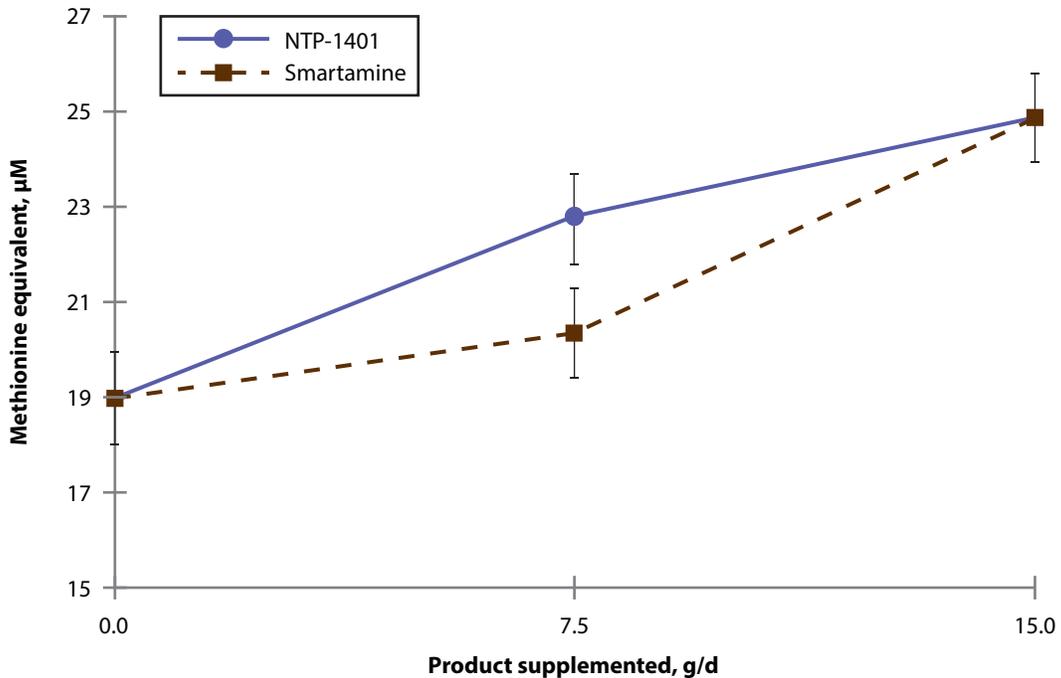


Figure 6. Effects of supplemental NTP-1401 and Smartamine on plasma concentrations of total methionine equivalents (sum of L-methionine, D-methionine, and methionine hydroxy analog). Linear effect of methionine supplementation ( $P < 0.0001$ ); quadratic effect of methionine supplementation ( $P = 0.63$ ); differences between sources ( $P = 0.13$ ); and source  $\times$  level interaction ( $P = 0.14$ ).