1539 (M253) Changes in plasma methionine concentrations after administration of two different doses of rumen protected methionine.

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Feeding rumen-protected limiting amino-acids, such as methionine (MET), to dairy cows may allow feeding of diets with lower amounts of crude protein while increasing milk protein and feed efficiency. Information on the changes of circulating MET concentrations after feeding may provide valuable information on both its usage and its metabolism, which could then be used by field nutritionists. The objective of the present experiment was to determine changes in plasma MET concentrations after administration of a single bolus of rumen-protected methionine (RPM). Non-lactating, non-pregnant dairy cows (n = 16) weighing 694 ± 16 kg were randomly assigned to three treatments: 1) untreated control (n = 4); 2) bolus containing 10 g of RPM (Smartamine; 6 g of metabolizable MET; n = 6); and 3) bolus containing 20 g of RPM (Smartamine; 12 g of metabolizable MET; n = 6). Blood samples were collected at 12h before treatment, immediately before treatment, and at 6, 12, 18, 24, 36, and 48h after treatment. Plasma was assayed for free amino acid by gas chromatography using a commercial kit (EZ:faast-GC-FID Physiological, Phenomenex). Data were analyzed by repeated measures using the PROC MIXED of SAS. Plasma MET concentrations tended to differ among treatments (P = 0.08) and were greater for cows receiving the 20 g bolus, intermediate for cows receiving the 10 g bolus, and least for control cows (peak average 57.5 ± 0.8μM, 26.9 ± 0.2μM, and 20.3 ± 0.2μM, respectively). Before treatment, all cows had low MET concentrations (21.4 ± 0.5μM), and MET concentrations remained low throughout the experimental period in controls. At 12 and 18h, MET concentrations increased (P = 0.09) by 30% in cows receiving the 10 g bolus (26.5 ± 0.2μM) compared to control cows (20.3 ± 0.1μM); however, cows receiving the 20 g bolus increased more than 100% (50.4 ± 4.1μM) and were greater than either controls (P < 0.01) or cows treated with the 10 g bolus (P < 0.01). By 24 h after treatment, MET concentrations differed among treatments (P < 0.001) and were least for controls (22.7 ± 0.1μM), intermediate for cows receiving the 10 g bolus (23.7 ± 0.1μM), and greater for cows receiving the 20 g bolus (29.6 ± 0.1μM). Methionine concentrations did not differ among treatments (P = 0.85) at 36 and 48 h. Lysine concentrations did not differ among treatments (P = 0.52) and were 143.3 ± 5.2μM, 133.3 ± 5.4μM, and 139.6 ± 6.8μM for controls, cows receiving 10 g of RPM, and cows receiving 20 g of RPM, respectively. In conclusion, plasma MET concentrations were affected by treatment dose and time after treatment. Time after treatment should be considered when evaluating effectiveness of MET supplementation. *Supported by Hatch project WIS01240 and Adisseo USA, Inc.*

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1540 (M254) A three-step in vitro procedure for evaluating rumen-protected lysine products.

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The three-step in vitro procedure is used for estimating intestinal digestibility of the RUP fraction of feedstuffs. However, rumen protected amino acid products have been evaluated in various ways for estimates of bioavailability. The objective of this study was to propose a three-step in vitro procedure for rumen-protected Lysine products (RPL), which is composed of buffer solutions with enzymes and can be developed as a standardized method to evaluate RPLs. Three grams of six different RPLs were weighed into nylon bag (5 × 7 cm, pore size 53 ± 10 μm). Bags were incubated using a dissolution apparatus for drug evaluation with rotating paddles at 100 rpm at 39°C. Three individual vessels were allocated to each RPL as ruminal, abomasal and duodenal phases, respectively. Modified McDougal's buffer containing lipase (900 mL) was used to simulate ruminal conditions (pH 6.8). A hydrochloride buffer containing pepsin (900mL, pH 2.0) and a phosphate buffer containing pancreatin and gall powder (900 mL, pH ≈ 7.9) were used to simulate abomasal and intestinal conditions, respectively. After a 20-h incubation in ruminal vessels, aliquot samples of solution were taken, and bags containing each RPL were transferred from ruminal to abomasal vessels. These procedures were repeated after a 2-h incubation followed by incubation in duodenal vessels for 8 h; aliquot buffer samples were taken again. Each buffer sample was analyzed for Lys, and amount of lysine escaping dissolution was calculated by subtracting buffer lysine from original feed lysine provided to the assay. Residual Lys under ruminal conditions was compared with extent of in situ ruminal protection. Statistical differences (P < 0.05) were tested by a one-way ANOVA. In vitro ruminal protection measured by this procedure correlated (P < 0.05) with in situ ruminal protection with a correlation coefficient of > 0.9. The RPLs showed various characteristics; high/medium/low ruminal protection and high/low post-ruminal Lys release. However, when pH of the ruminal buffer was reduced, one of the RPLs showed an increase (P < 0.05) in ruminal protection using in vitro procedures (46.2 ± 9.3% at pH 6.8, 90.0 ± 4.0% at pH 6.2, n = 3). Results from this study indicate that a buffer-based three-step in vitro procedure can be a useful tool to evaluate RPLs, but further research is needed to optimize pH of ruminal conditions.