Methionine Supplementation Options

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Introduction

Methionine (Met) and lysine (Lys) have been identified most often as the two most limiting amino acids (AA) for lactating dairy cows. This is largely because of their low concentrations in feed protein as compared to their concentrations in milk and ruminally synthesized bacterial protein. The NRC (2001) suggested concentrations of Lys and Met in metabolizable protein (MP) for maximal use of MP for milk protein production are 7.2 and 2.4%, respectively. Under almost all circumstances, these concentrations cannot be achieved, and as a result, "practical recommendations" for Lys and Met in MP of 6.6 and 2.2% are being used by the authors (Schwab et al., 2003). These concentrations can generally be achieved in corn-based rations by using a combination of high-Lys protein supplements (e.g., blood, fish, and soybean meals) and a rumen-protected Met (RPMet) product and limiting intake of rumen-undegraded intake protein (RUP) to requirement levels. Not using a RPMet product requires the mix-and-matching of protein supplements to achieve the desired Lys:Met ratio in MP of 3.1 and as a result, lowers the concentrations of both Lys and Met in MP that are achievable (Schwab et al., 2003)

The purpose of this paper is to review the Met products that have been suggested to provide Met to ruminants. These include RPMet products and two forms of 2-hydroxy-4-methylthio butanoic acid (HMB). A description of the products and methods for determining their metabolic availability to the host animal are reviewed.

Ruminally Protected Methionine

History of Development

Interest in protecting free Met from ruminal degradation dates back to the 1960's and early 1970's when it became apparent from abomasal, intestinal, and intravenous infusion trials that the profile of absorbed Met was not always optimum in ruminants (Chalupa, 1975; Schwab, 1995). These trials indicated that the sulfur AA were clearly first limiting for wool growth and body weight gains of sheep and that Met was a limiting AA for growing cattle and lactating dairy cows. Thus, several laboratories began to devise procedures to protect Met from ruminal degradation (Chalupa, 1975). Subsequent interest developed in protecting Lys when it was discovered to be second limiting for growing lambs and either first or second limiting for growing cattle and lactating dairy cows.

Many approaches have been evaluated to physically protect Met and Lys from ruminal degradation. Initial attempts focused on protecting Met with lipids, often in combination with inorganic materials and carbohydrates as stabilizers, softening agents and fillers. For example, in the 1960's Delmar Chemicals of Canada developed a product in which a core of DL-Met, colloidal kaolin, and tristearin was wrapped in a film of tristearin. The product contained 20% Met. A few years later, Rumen Kjemi a/s of Oslo, Norway introduced a somewhat more efficacious product (Ketionin®) that had a higher intestinal release of the encapsulated Met. The product contained 30% DL-Met, 2% glucose, 4% stabilizing, antioxidant, and flavoring agents, 6% $CaCO_3$, and 58% tristearin and oleic acid.

Several other lipid protected Met products have also been evaluated (Loerch and Oke, 1989; Schwab, 1995). The greatest challenge with using lipids as the primary encapsulating material is to identify a combination of materials and process that had both a high ruminal escape and intestinal release of Met.

The most effective approach to date has been to surface-coat Met with enzyme-resistant, pH-sensitive synthetic polymers that are insoluble in the more neutral pH environment of ruminal digesta but highly soluble in the acidic abomasum. This approach provides for a post-ruminal delivery system that is independent of enzyme function and, instead, relies on the pH differences between the rumen and the abomasum for ruminal protection and intestinal release. Polymer-protected Met has higher ruminal protection and intestinal release coefficients than other products. The patent rights for the use of pH-sensitive polymers for protecting nutrients from ruminal degradation is currently held by Adisseo, Inc., Antony France.

Another method that has been explored for increasing supplies of Met to ruminants is the use of analogs and derivatives of Met. Amino acid derivatives are free AA to which a chemical blocking group has been added to the α-amino group or in which the acyl group has been modified. Some examples of Met derivatives that have been shown to have some resistance to ruminal degradation are isopropyl-DL-Met, t-butyl-DL-Met, N-stearoyl-DL-Met, N-oleoyl-DL-Met, and capryl-caproylic-DL-Met (Loerch and Oke, 1989). There is evidence that extent of ruminal escape is greater with shorter chain rather longer chain alkyl esters. Although these and other derivatives show promise, most have not been investigated adequately to determine the extent to which they will increase post-ruminal supplies of absorbable Met.

Amino acid analogs are generated from the substitution of the α-amino group of the AA with a non-nitrogenous group such as a hydroxyl group. The most studied AA analog is Met hydroxy analog (MHA; DL-α-hydroxy-γ-mercaptobutyrate) or more appropriately called 2-hydroxy-4-methylthio butanoic acid (HMB). Studies indicate that free HMB is more resistant to ruminal

degradation than free Met, that it can be absorbed from the rumen and omasum through passive diffusion, and that ruminants have the enzymes for the conversion of HMB to Met. However, because of observed minimal effects on blood Met concentrations and milk protein concentrations when fed to lactating dairy cows fed Met-deficient diets, it appears that its ability to substitute for absorbed Met in dairy cows is minimal.

Recent research has shown that several esters of HMB enhance ruminal escape of HMB, at least in part because of their apparent ability to be absorbed across the rumen wall. The isopropyl ester of HMB has been shown to have an excellent replacement value for absorbed Met (Robert et al., 2001b, Schwab et al., 2001).

Commercial RPMet Products

Met-Plus™ (Nisso America, Inc.). This is an example of a lipid-protected product. It is a matrix compound that contains 65% DL-Met embedded in a mixture of calcium salts of long-chain fatty acids, lauric acid, and butylated hydroxytoluene (BHT); BHT is a preservative for the fatty acids. However, like other lipid-coated products, the technology relies on achieving a balance between ruminal protection vs. intestinal release so as to maximize the amount of Met available for intestinal absorption while minimizing losses in the rumen and in feces.

Mepron® M85 (Degussa Corporation, Germany). This is an example of a surface-coated, carbohydrate-protected product. The small pellets have a diameter of 1.8 mm, a length of 3-4 mm, and an approximate density of 1.2 g /cm^3. The pellets consist of a core of Met and starch coated with several thin layers of ethylcellulose and stearic acid. The final product contains a minimum of 85 % DL-Met, and approximately 8.5% non-structural carbohydrates, 3.5% NDF, 1.5 % ash, 1.0 % moisture, and 0.5% crude fat. The technology is a combination of coating materials and application that allows for a large payload of Met. Because enzymatic digestion of the ethyl cellulose is minimal, degradation of the product occurs primarily through physical action and abrasion. The result is a product that results in a slow degradation in the rumen and a slow release of Met in the intestine.

Smartamine™ M (Adisseo, Inc., Antony France). This is an example of a lipid/pH-sensitive polymer-protected product. It is a surface-coated product that contains a minimum of 75% DL-Met. The small 2-mm pellets consist of a core of DL-Met plus ethylcellulose which is covered with a coat of stearic acid containing small droplets of poly (2-vinylpyridine-co-styrene). The copolymer contributes 3% by weight of the final product. The presence of the copolymer appears to alter the stereochemistry of the stearic acid such that the surface-coating becomes enhanced in its resistant to ruminal degradation. The presence of the copolymer, as a result of its solubilization at low pH, also allows for rapid release of the Met in the abomasum.

Methionine Analogs

Background

Koenig et al. (1999) reported that 50% of HMB escaped ruminal degradation and became available for postruminal absorption in early lactation dairy cows. The approach was to feed the cows a pulse-dose of 90 g of Alimet (an 88% aqueous solution of HMB, Novus International, Inc. St. Louis, MO) and 600 ml of Cr-EDTA, a liquid phase marker, mixed with 2 kg of ground corn grain. Ruminal escape of HMB was calculated from the fractional rate constants for ruminal disappearance of HMB and passage of liquid. Subsequent studies using a dual effluent continuous culture system indicated ruminal escape values of 22 to 43% for HMB (Vázquez-Añón et al. (2001). Other research has shown that HMB can be absorbed across ruminal and omasal epithelium (McCollum et al., 2000), and that if absorbed, it can be converted to Met (Belasco, 1972, 1980; Papas et al., 1974; Wester et al., 2000a,b). These findings led to the use of HMB as a substitute for RPMet for lactating dairy cows.

However, the extent to which dietary HMB substitutes for absorbed Met for milk protein production in lactating cows remains questionable. Many studies have indicated that content of protein in milk is sensitive to adequacy of Met in MP and that milk protein percentage increases when the content of Met in MP is improved (Guinard and Rulquin, 1995; Schwab et al 1976,1992; NRC, 2001; Pisulewski et al, 1996). Yet, supplementing apparent Met-deficient diets with HMB did not increase milk protein concentrations (Ellis, 1986; Ellis et al., 1986; Johnson et al., 1999; Rode et al., 1998). Moreover, supplementing diets with HMB had either little or no effect on blood Met concentrations (Johnson et al., 1999; Papas et al., 1974; Polan et al., 1970; Robert et al., 1997). These observations question the use of HMB as a substitute for RPMet that are fed to achieve a desired concentration of Met in MP and to increase supplies of Met to the mammary gland.

More recently, it has been demonstrated that the isopropyl ester of HMB (HMBi) is considerably more effective than HMB as a source of MP-Met for lactating cows (Robert et al., 2001a,b). The researchers reported that HMBi was 56% as effective as Smartamine M™ in increasing blood Met concentrations. The two sources of metabolizable Met were given as a pulse-dose in equimolar amounts (49 g of Met equivalents) to non-lactating cows fed 8.5 kg/d of DM. In previous work using a similar blood response technique, the same workers reported that HMB was only 3% as effective as Smartamine M™ in increasing blood Met concentrations (Robert et al., 1997).

Similar results were obtained in a recent study conducted at the University of New Hampshire. Schwab et al. (2001) estimated that HMBi was 53% as effective as Smartamine M™ in increasing milk protein percentages. In order to

estimate the "Met bioavailability" of HMB and HMBi for lactating cows, dose response titrations were carried out using milk true protein content as the response criteria. Four Met sources were used: 1) Smartamine M™, 2) HMB, 3) HMBi, and 4) a combination of 1/3 HMB and 2/3 HMBi (HMB/HMBi). Treatment levels were (g Met equivalents/d per 25 kg of DMI): Smartamine M™ (0, 10, 15, 20, and 25), HMB and HMBi (0, 15, 20, 25, and 30), and HMB/HMBi (0/0, 5/10, 8.3/16.7, 11.7/23.3, and 15/30). Milk protein concentrations increased with all treatments except HMB. The corrected milk protein percentages were: Smartamine M™ (2.99, 3.08, 3.15, 3.15, and 3.13; quadratic effect), HMB (3.04, 3.02, 3.03, 3.06, and 3.03), HMBi (3.05, 3.11, 3.16, 3.17, and 3.19; linear effect), and HMB/HMBi (3.07, 3.13, 3.12, 3.16, and 3.18; linear effect). Based on differences of slope, it was calculated that HMB was 53% as effective as Smartamine M™ and that the HMB/HMBi combination was 43% as effective as Smartamine M™. The results of the experiment indicated that HMB provided little or no Met for milk protein synthesis. However, both Smartamine M™ and HMBi were effective at providing post-ruminal Met as evidenced by the increase in milk protein concentration.

Commercial Products

Alimet® (Novus International, Inc. St. Louis, MO, USA) and **Rhodimet™ AT88** (Adisseo, Inc., Antony France). Both are liquid sources of HMB. Chemically, the compounds are the same and both are used extensively as a substitute for methionine in the poultry and swine industry. Novus International also has Alimet® patented for use in dairy cows and recommends its use as a source of RPMet.

Efficacy of Methionine Products

Responsible use of RPMet products and Met analogs as Met supplements requires estimates of their ability to provide (or spare) absorbed Met. Insofar as possible, estimates of "Met bioavailability" must be accurate and reliable under the conditions in which they are fed.

Approaches

Unfortunately, there is no universally accepted, standardized procedure(s) for obtaining estimates of Met bioavailability. These are needed to bring uniformity to estimates of Met bioavailability and to more accurately compare the efficacy of different products. Current approaches can be categorized as factorial approaches, blood response approaches, and production response approaches.

The factorial approach involves independent measurements of ruminal escape, intestinal disappearance (digestibility), and in the case of free or protected forms of HMB, metabolic conversion to Met. Animals with cannulae in the rumen, duodenum, and preferably also in the ileum, are required. Estimates of

ruminal escape and intestinal disappearance of Met from RPMet products have been obtained using both the in situ, nylon-bag procedure and the cannulated cow in vivo procedure. Use of the in situ procedure requires measurements of rate of passage of RPMet products from the rumen. The in situ procedure is not suitable for soluble products such as HMB and HMBi.

Blood response approaches are an attractive alternative to factorial approaches because they are easier to conduct and they allow liquid and pulverulent products to be evaluated. Studies with cattle have shown that a linear relationship exists between increasing supplies of absorbable Met and plasma Met concentrations. Two variations of the approach have been used. The first is the dose-response approach that involves determination of differences in slope of measured blood Met concentrations between graded dietary doses of the product and graded intestinal doses of infused methionine. The second is the "area under the curve" (AUC) approach. This approach involves the ruminal administration of a pulse-dose of equimolar amounts of different Met sources and comparing the AUC of the plasma Met response curves that result. To obtain estimates of Met bioavailability for Met products, Smartamine™ M has been used as the positive control treatment with the assumption that it has a Met bioavailability value of 80%.

A production response approach has recently been used to obtain estimates of the Met bioavailability values for free HMB and HMBi (Schwab et al., 2001). This approach, as previously described, involved determination of differences in slope of measured milk protein concentrations between graded dietary doses of the HMB products and Smartamine™ M when cows were fed a Met-deficient diet. Numerous studies with lactating cows indicate that content of milk protein in milk is more responsive than milk yield to small changes in concentrations of Met in MP.

A Comparison of Some Available Products

Of the products described in this paper, Smartamine™ M appears to have the greatest efficacy as a source of absorbable Met. Nylon bag studies indicate ruminal stability exceeding 90% at 24 h and intestinal release values approximating 90% (determined either by the amount released after 1 h in a pH 2.0 HCL-pepsin solution or by the mobile bag technique after exposure to the HCL-pepsin solution). A cannulated cow in vivo experiment involving early lactation cows indicated ruminal escape values that averaged 90% across four different diets and an average intestinal disappearance value of 98%, resulting in an average Met bioavailability value of 88% (Robert and Williams, 1997).

Several studies using the different approaches mentioned above, plus the use of in vitro studies, indicate that the efficacy of Mepron® M85 as a source of absorbable Met is intermediate between that of Smartamine™ M and lipid protected products. Mepron® M85 has been shown to be degraded faster in the

rumen than Smartamine™ M and result in significantly smaller increases in plasma sulfur AA concentrations than Smartamine™ M. A summary of in situ nylon experiments indicates ruminal protection values that approximate 90% at 2 h, 85% at 6 h, 70% at 12 h, 60% at 24 h, and 15% at 96 h. Estimates of passage rate from the rumen, which have not been reported, are needed to calculate ruminal escape. Mepron® M85 also is less digestible in the small intestine than Smartamine™ M. Use of the mobile bag technique has indicated that 25-70% of the Mepron® M85 entering the small intestine is excreted in the feces, with larger amounts being excreted with higher feed intakes. It appears questionable as to whether the mobile bag technique is an appropriate method for measuring the intestinal release of Met from a RPMet product such as Mepron® M85 that relies on abrasion and physical forces for its degradation.

Of the Met analogs, as already discussed, only HMBi appears to provide significant quantities of Met to the lactating dairy cow.

Summary and Conclusions

Until HMBi becomes available, the Met-source options for increasing supplies of metabolizable Met to lactating cows are the three described RPMet products. The use of these products along with selective use of protein supplements, give dairy nutritionists the opportunity to more adequately balance diets for Lys and Met.

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