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Meta-analysis of 2-hydroxy-4-methylthio-butanoic acid supplementation on ruminal fermentation, milk production, and nutrient digestibility

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ABSTRACT

Methionine is considered one of the most important essential AA for milk protein synthesis in dairy cows. Supplementation of unprotected, free Met is nearly 100% degraded by ruminal microorganisms, which complicates supplementation. 2-Hydroxy-4-methylthio-butanoic acid (**HMTBa**) can be converted to Met in the body and is used as a Met source in dairy production. However, results of published studies assessing the effects of supplementing Met sources, including HMTBa, on performance variables are inconsistent. A meta-analysis was performed to quantitatively summarize the accumulated results of HMTBa supplementation on animal performance and nutrient digestibility. Data pertaining to HMTBa dose, dietary composition, and major performance variables (rumen volatile fatty acid composition, milk production, nutrient digestibility) were collected from 39 articles containing 169 treatment means. Publications were from scientific journals published from 1970 to 2018; 1 internal report from Novus International Inc. (St. Charles, MO) was also included. The HMTBa effects on response variables were analyzed using linear mixed models with random study effects. Other explanatory variables tested included neutral detergent fiber and crude protein percent as well as days in milk. Results showed that HMTBa supplementation increased blood Met concentration and milk fat yield but had no effect on nutrient digestibility.
Key words: 2-hydroxy-4-methylthio-butanoic acid (HMTBa), meta-analysis, digestibility

INTRODUCTION

Methionine is often a limiting AA for milk protein synthesis (Vyas and Erdman, 2009; Patton, 2010). In addition to its role as a substrate for protein synthesis, Met is a strong regulator of protein synthesis (Appuhamy et al., 2011) and is involved in transsulfuration and methylation reactions (Brosnan and Brosnan, 2006). Balancing rations with supplemental Met to improve the AA profile could allow for reduced dietary CP, maximizing lactation performance, and minimizing N excretion to the environment.

Free Met in an unprotected form is almost completely degraded by rumen microbes, resulting in minimal or no additional postruminal Met supply. An effective way to increase postruminal Met supply is to feed a Met product that escapes microbial degradation in the rumen. Protection is commonly achieved by chemical modifications or by physically protecting the Met. One example of chemical modification is 2-hydroxy-4-methylthio-butanoic acid (**HMTBa**), which is a Met analog where the amino group is replaced with a hydroxyl group. The results on whether this modification could effectively protect HMTBa from ruminal degradation are inconsistent. Koenig et al. (1999) reported about 50% of the HMTBa escaped ruminal degradation and became available for postruminal absorption, whereas Jones et al. (1988) observed 99% of the HMTBa was altered or degraded by rumen microbes.

Researchers have conducted numerous studies over the years to investigate the effects of HMTBa supplementation on performance of dairy cows, but results have been highly variable. Some studies have reported HMTBa supplementation increased milk fat yield (Polan et al., 1970; Lundquist et al., 1985) and enhanced rumen microbial activity (Holter et al., 1972; Lee et al., 2015). Some studies have also reported no beneficial effects of supplemental HMTBa (Olson and Grubaugh, 1974; Wallenius and Whitchurch, 1974). Because of this disparity in the literature, a need and an opportunity exist to test the effects of supplementing HMTBa across all published studies. The meta-analysis done by Zanton et al. (2014b) included 17 papers on HMTBa supplementation, but only lactation responses to supplemental Met sources were analyzed. Their meta-analysis concluded HMTBa supplementation increases

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milk fat yield by 45 g/d and milk protein yield by 13 g/d, suggesting beneficial effects of supplemental HMTBa. Combining previous work and the previous meta-analysis, these data suggest a potential for HMTBa to affect rumen fermentation. More specifically, with increased ruminal microbial activity from supplemental HMTBa, it is possible HMTBa would increase nutrient digestibility and microbial N flow. Thus, a more comprehensive meta-analysis is needed to investigate the effects of supplemental HMTBa on rumen fermentation, milk production, and nutrient digestion.

The objective of this work was to use a meta-regression approach to study the effects of HMTBa supplementation. We hypothesized that supplementation of HMTBa would affect ruminal fermentation, milk production, and nutrient digestibility.

MATERIALS AND METHODS

Data Collection and Preparation

To accomplish the objective, data were collected from different published articles in the literature (see Appendix Table A1 for list of publications). PubMed, Science Direct, AGRICOLA, and Web of Science databases were searched using the keywords combination from group one (methionine, methionine hydroxyl analog or MHA, 2-hydroxy-4-methylthio-butanoic acid or HMTBa or HMB, and ALIMET feed supplement) and group 2 (ruminants, dairy cows, cows, or cattle). Literature cited in these papers was also used to look for papers investigating HMTBa effects. A total of 39 articles reporting results from a total of 169 treatments were collected. Among the 39 articles, 3 reported work conducted with beef cattle breeds and the remainder reported findings from dairy cows. Articles that did not report the inclusion rate of HMTBa were not used. Data on dietary nutrient composition, HMTBa dose, DIM, milk yield, milk composition, ruminal VFA concentration, and blood Met concentration were collected from each article. Milk fat and protein yield were calculated using milk yield and fat or protein content when component yields were not reported. Ruminal VFA were converted to a percentage of total VFA, where total VFA was the sum of acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate if reported. The supplemental amount of commercial HMTBa was converted to a pure HMTBa basis using multipliers of 0.88 and 0.84 for liquid and Ca salt forms of HMTBa, respectively. As a categorical variable, DIM at the beginning of the study was grouped into 4 categories (0 = dry period; 1 = first third of lactation, where DIM is less than 100 d after calving; 2 = second third of lactation, where DIM is between 100 and 200 d after calving; 3 = last third of lactation, where DIM is more than 200 d after calving).

Model Derivation Procedure

All statistical analysis were conducted using R v. 3.1.3 (R Core Team, 2015). The lmerTest package (Kuznetsova et al., 2016) was used to derive linear mixed models with random intercept effects for each study. Independent variables used in the model were HMTBa dose (g/d), CP percent, NDF percent, and DIM category. Initial models generally included all independent variables described above as potential effectors of the response variable. Interactions of HMTBa and each independent variable, as well as quadratic effects of HMTBa, were tested but not significant, and thus were not included in the initial models. Models were derived following the procedure outlined in Roman-Garcia et al. (2016) and White et al. (2016) with minor modification. Briefly, final models were derived using backward elimination of nonsignificant effects, with the parameter possessing the largest P-value removed from the model in the subsequent round. The process was repeated until all P-values were less than 0.1. Any remaining parameters with variance inflation factors (**VIF**) greater than 10 were removed. The VIF is a measure of the severity of multicollinearity reflecting the degree to which parameter estimate variance is inflated due to collinearity and has been used in previous work as a model selection tool (White et al., 2017). A VIF >10 is often used to indicate unacceptably high multicollinearity (Roman-Garcia et al., 2016) and is the threshold that we used in the current study.

Evaluating Model Performance

Overall variance of the predictions was determined by calculation of concordance correlation coefficients (**CCC**; St-Pierre, 2004)) and mean square error (**MSE**) as described by Bibby and Toutenburg (1977):

$$\mathrm{MSE} = \frac{1}{n}\sum_{i=1}^{n}\left(O_i - P_i\right)^2,$$

where n represents the total number of observations, O_i the observed value, and P_i the predicted value. The MSE was decomposed into error due to random variation, deviation of the regression slope from unity (slope bias), and central bias (mean bias). Root mean square errors (**RMSE**) were obtained by taking the square root of the MSE. This RMSE was expressed as a proportion of the observed mean to estimate the overall prediction error. Inclusion of the random intercept effects for each