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Predicting omasal flow of nonammonia N and milk protein yield from in vitro-determined utilizable crude protein at the duodenum

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ABSTRACT

This study evaluated the relationship between utilizable crude protein (uCP) at the duodenum estimated in vitro and omasal flow of crude protein (CP; omasal flow of nonammonia N × 6.25) measured in lactating dairy cows. In vivo data were obtained from previous studies estimating omasal digesta flow using a triple-marker method and ^{15}N as microbial marker. A total of 34 different diets based on grass and red clover silages were incubated with buffered rumen fluid previously preincubated with carbohydrates for 3 h. The buffer solution was modified to contain 38 g of $NaHCO_3$ and 1 g of $(NH_4)HCO_3$ in 1,000 mL of distilled water. Continuous sampling of the liquid phase for determination of ammonia-N was performed at 0.5, 4, 8, 12, 24, and 30 h after the start of incubation. The ammonia N concentrations after incubation were used to calculate uCP. The natural logarithm of uCP [g/kg of dry matter (DM)] at time points 0.5, 4, 8, 12, 24, and 30 h of incubation was plotted against time to estimate the concentration of uCP (g/kg of DM) at time points 16, 20, and 24 h using an exponential function. Fixed model regression analysis and mixed model regression analysis with random study effect were used to evaluate the relationships between predicted uCP (supply and concentration) and observed omasal CP flow and milk protein yield. Residual analysis was also conducted to evaluate whether any dietary factors influenced the relationships. The in vitro uCP method ranked the diets accurately in terms of total omasal CP flow (kg/d) or omasal CP flow per kilogram of DM intake. We also noted a close relationship between estimated uCP supply and adjusted omasal CP flow, as demonstrated by a coefficient of determination of 0.87, although the slope of 0.77 indicated that estimated uCP supply (kg/d) was greater than the value determined in vivo. The linear bias with mixed model analysis indicated that uCP supply overestimated the difference in omasal CP flow between the diets within a study, an error most likely related to study differences in feed intake, animals, and methodology. Predicting milk protein yield from uCP supply showed a positive relationship using a mixed model (coefficient of determination = 0.79), and we observed no difference in model fit between the time points of incubation (16, 20, or 24 h). The results of this study indicate that the in vitro method can be a useful tool in evaluating protein value of ruminant diets.

Key words: protein evaluation, protein degradation, metabolizable protein, dairy cow

INTRODUCTION

Protein evaluation systems aim to optimize the feeding of protein, and accurate prediction of the protein requirement of the animal is a prerequisite of a functioning evaluation system (Schwab et al., 2005). Since the 1970s, when the MP systems were established, they have become commonly used and increasingly accurate in predicting production responses and particularly milk protein yield (**MPY**). Metabolizable protein is defined as AA absorbed in the small intestine and it consists of microbial protein, RUP, and a small fraction of endogenous AA. Several systems for calculating MP have evolved (GfE, 2001; NRC, 2001; Volden, 2011). Microbial (bacterial) MP is calculated as a function of digestible OM, with or without discounting substrates that provide little or no energy for microbes.

The procedures to determine ruminal CP degradability, and hence supply of RUP to the small intestine, are most often based on the in situ bag procedure (NRC, 2001; Volden, 2011). Several problems exist with the use of the in situ technique when measuring ruminal protein degradability (Michalet-Doreau and Ould-Bah, 1992; Broderick and Cochran, 2000), and the technique has proven difficult to standardize, as indicated by large between-laboratory differences in ruminal protein degradability values (Madsen and Hvelplund, 1994). The major problems causing bias in the procedure

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are the assumption that the soluble protein fraction is completely degradable, poor bag porosity preventing microbes from entering the bag, and microbes colonizing the bag residues (Broderick et al., 2010).

Broderick et al. (2010) found a strong relationship between NAN flow observed at the omasal canal and total CP flow from the rumen predicted by the NRC (2001) model. However, evaluation of the NRC model indicated a 26% slope bias in the RUP supply, suggesting that the system overestimated the range of RUP supply (Broderick et al., 2010). Those authors also speculated that the overestimation derives from problems with the in situ procedure. Santos et al. (1998) performed a meta-analysis studying the effects of replacing soybean meal with high-RUP sources and found that milk yield increased only in 17% of the comparisons. Similarly, Ipharraguerre and Clark (2005) found that mean milk production responses to RUP supplements were negligible compared with the response to solvent-extracted soybean meal. In both analyses (Santos et al., 1998; Ipharraguerre and Clark, 2005), there were indications of reduced microbial protein synthesis with RUP supplementation. These previous studies indicate what was stated by Lebzien and Voigt (1999), that the current protein evaluation systems may overemphasize the importance of RUP supply to the dairy cow and that calculated microbial CP and RUP may not be completely additive.

The modified Hohenheim gas test (Steingaß et al., 2001; Steingaß and Südekum, 2013) offers an in vitro method that simplifies the aim and can eliminate some methodological inaccuracies of modern protein evaluation systems. The method involves incubation of feeds with rumen fluid, after which NH_3-N is measured. The NAN content is used to calculate utilizable crude protein (**uCP**) at the duodenum, which corresponds to ruminal microbial CP and RUP flowing to the duodenum. Edmunds et al. (2012) used the modified Hohenheim gas test to validate forage protein values against the German feed protein evaluation system (GfE, 2001) and reported that the method has high potential for estimating uCP. Theoretically, the problems of the in situ method (particle loss, soluble N, microbial contamination) should be smaller in the uCP method, which also takes into account possible effects on microbial N synthesis.

Our hypothesis was that the in vitro-determined uCP would predict omasal flow of CP (omasal flow of NAN $\times$ 6.25) determined in dairy cows fed different diets. By incubating the whole diets instead of single feeds, possible interactions between diet components and effects on microbial synthesis could be better taken into account. Our objective was to evaluate the relationship between uCP estimated in vitro and omasal CP flow measured in cannulated cows fed a wide range of diets. An additional objective was to determine the relationship between in vitro uCP concentration and MPY.

MATERIALS AND METHODS

Animal studies conducted in Sweden were registered and conducted according to guidelines approved by the Swedish University of Agricultural Sciences Animal Care and Use Committee and the National Animal Research Authority. Animal studies conducted in Finland were managed according to legislation documented within the Finnish Animal Welfare Act (247/96) and the Order on Using Vertebrate Animals for Scientific Purposes (1076/85; Ministry of Agriculture and Forestry, 1996).

Experimental Design and Animals

Thirty-four diets were evaluated in vitro using rumen fluid inoculum. Each diet was randomly distributed within and between 4 runs, resulting in 4 observations per diet. In each run, 2 blanks were incubated.

Rumen fluid for in vitro inoculum was collected from 3 lactating Nordic Red cows fitted with 10-cm ruminal cannulas (Bar Diamond Inc., Parma, ID). The cows were fed diets consisting of grass silage, crimped barley, canola meal, and minerals (55, 34, 10, and 1% on DM basis, respectively). Milking was performed 2 times daily, at 0600 and 1500 h.

Origin of Samples

Thirty-four diets from 8 different in vivo omasal flow studies (Table 1) conducted at the MTT Agrifood Research Finland (now Natural Resources Institute Finland, Luke, Helsinki, Finland) and the Swedish University of Agricultural Sciences were evaluated in vitro. Dietary ingredients within a study were pooled over experimental periods, and TMR samples for in vitro runs were prepared in the same proportions as fed to the animals.

All production studies used ruminally cannulated lactating dairy cows in complete and incomplete Latin square designs. Experimental diets were typical for northern Europe, based on grass and red clover silages from different harvest regimens, supplemented with a variety of grains and protein feeds (Table 1). Descriptive data on the in vivo studies (Table 2) showed mean values of 19.6 kg of DMI/d and 27.7 kg of ECM/d. In all studies, omasal digesta was collected according to the sampling technique by Huhtanen et al. (1997) with the modification of Ahvenjärvi et al. (2000), placing a collection tube through the rumen cannula by the