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Ruminal metabolism of grass silage soluble nitrogen fractions

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ABSTRACT

The present study was conducted to investigate ruminal N metabolism in dairy cows using ^{15}N-labeled N sources and dynamic models. The data summarized in this study were obtained from 2 of 4 treatments whose effects were determined in a 4 × 4 Latin square design. Soluble N (SN) isolated from timothy grass silage labeled with ^{15}N and ammonia N (AN) labeled with ^{15}N were administered into the rumen contents of 4 ruminally cannulated dairy cows. Ruminal N pool sizes were determined by manual evacuation of rumen contents. The excess ^{15}N-atom% was determined in N-fractions of rumen digesta grab samples that were collected frequently between 0 to 72 h and used to determine ^{15}N metabolism in the rumen. Calculations of area under the curve ratios of ^{15}N were used to estimate proportions of N fractions originating from precursor N pools. A model including soluble nonammonia N (SNAN), AN, bacterial N, and protozoal N pools was developed to predict observed values of ^{15}N atomic excess pool sizes. The model described the pool sizes accurately based on small residuals between observed and predicted values. An immediate increase in ^{15}N enrichment of protozoal N suggests physical attachment of bacteria pool to protozoa pool. The mean proportions of bacterial N, protozoal N, and feed N in rumen solid phase were 0.59, 0.20, and 0.21, respectively. These observations suggest that protozoal N accounted for 0.25 of rumen microbial N. About 0.90 of the initial dose of AN was absorbed or taken up by microbes within 2 h. Faster ^{15}N enrichment of bacterial N with SN than with AN treatment indicates a rapid adsorption of SNAN to microbial cells. Additionally, the recovery of ^{15}N as microbial and feed N flow from the rumen was approximately 0.36 greater for SN than for the AN treatment, indicating that SNAN was more efficiently used for microbial growth than AN. The present study indicated that about 0.15 of microbial N flowing to the duodenum was of protozoal origin and that 0.95 of the protozoal N originated from engulfed bacterial N. The kinetic variables indicated that 0.125 of SNAN escaped ruminal degradation, which calls into question the use of in situ estimations of protein degradation to predict the flow of rumen undegradable protein.
Key words: dairy cow, modeling, bacterial nitrogen, ammonia nitrogen

INTRODUCTION

Protein evaluation in ruminants is based on MP that is primarily composed of absorbed AA of microbial and feed origin. Most protein evaluation systems for ruminants (Sniffen et al., 1992; NRC, 2001; NorFor, 2011) rely on rate constants of ruminal protein degradation. Most commonly, ruminal in situ method has been used to assess ruminal protein degradability and RUP concentration of the feeds. In situ determinations of protein degradation have limitations, including (1) the assumption that proteins, peptides, and AA in the soluble fraction are completely degraded, (2) the physical restriction of feeds within the bag from microbial interaction and degradation, and (3) the imprecise quantification of microbial contamination of the undegradable residues (Broderick and Cochran, 2000). Theoretically, 1 unit of MP originating from microbial protein and RUP should have the same productive value. However, in a meta-analysis (Huhtanen and Hristov, 2009), regression coefficient of milk protein yield on bacterial MP was 5-fold greater compared with feed MP when the MP supply was estimated according to NRC (2001) using tabulated values. The difference in regression coefficients between the 2 sources of MP is partly related to the close association between energy intake and bacterial MP and to the more ideal AA profile of microbial MP compared with the more variable AA profile of digestible RUP. An analysis of omasal sampling data indicated that the differences in microbial MP between the diets were underestimated by the NRC (2001) system, whereas the differences in RUP were overestimated (Broderick et al., 2010).

Discrepancies between estimated MP supplies and observed production responses in dairy cows can also

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be related to underestimation of the MP value of soluble nonammonia nitrogen (**SNAN**) fractions. Plenty of evidence from studies using different methodologies indicates that SNAN fractions (free AA, peptides, and soluble proteins) can escape ruminal degradation (Chen et al., 1987; Hristov and Broderick, 1996; Reynal et al., 2007). Experimental evidence also indicates that peptides and free AA stimulate microbial protein synthesis (Argyle and Baldwin, 1989; Russell et al., 1992; Walker et al., 2005).

Dynamics of rumen N metabolism has been investigated using ^{15}N-labeled ammonia N (**AN**) and compartmental modeling (Aharoni and Tagari, 1991; Oldick et al., 2000). Labeling of forage N with ^{15}N is one of the techniques for studying metabolism of plant N fractions in the rumen. Hristov et al. (2001) used ^{15}N-labeled alfalfa silage and hay to investigate ruminal N metabolism of different N fractions. The method proved to be useful, but because of complicated transactions between different ruminal N pools, interpretation of the data was equivocal. Therefore, to avoid such problems inherent to labeled N sources with complex chemical composition, in the current study labeled grass silage was fractionated into soluble and insoluble fractions that were administered into the rumen.

The objective of the present study was to investigate the ruminal metabolism of AN and isolated N fractions from ensiled timothy grass. To achieve this objective, 4 treatments were allocated to experimental animals: ^{15}N-labeled AN (AN treatment), ^{15}N-labeled grass silage soluble N (**SN** treatment) and insoluble N fractions (**ISN** treatment), and ^{15}N-labeled casein (casein treatment). Timothy grass was labeled with ^{15}N during growth and preserved as formic-acid-treated silage. Subsequently, ensiled grass was fractionated into soluble (SN treatment) and insoluble N fractions (ISN treatment). Grass silage SN consisted of AN and SNAN fractions. The current paper reports only the ruminal metabolism of labeled AN and grass silage SN because the ruminal metabolism of ISN and casein treatments required further modifications to the current model and a full description of those results would have extended the paper considerably. It was hypothesized that grass silage SNAN fractions are not completely degraded in the rumen and that SNAN is a better N source for microbial protein synthesis than AN.

MATERIALS AND METHODS

Experimental Design and Diet Composition

Ruminal metabolism of 4 N sources labeled with ^{15}N was studied using 4 lactating Finnish Ayrshire dairy cows in a 4 × 4 Latin square with 14-d experimental periods. Multiparous cows (BW 660 kg, SD 40.1) were on average 96 DIM (SD 27.0) with a mean milk yield of 33.3 kg/d (SD 4.59) during the experiment. Throughout the study the animals received a diet consisting of 8.9 kg of DM/d of concentrate mixture and primary growth grass silage that was fed ad libitum 4 times daily at 0600, 0900, 1800, and 2000 h, allowing 0.05 to 0.10 for refusals. The daily concentrate portion was allocated to 4 equal meals offered simultaneously with grass silage. The concentrate mixture consisted of (as-fed basis) rolled barley, oats, sugar beet pulp, and rapeseed meal at 243 g/kg of each ingredient, 24 g/kg of commercial mineral premix (Viher-Minera Muro, Suomen Rehu Ltd., Helsinki, Finland), and 4 g/kg of calcium carbonate.

The chemical composition of grass silage, labeled grass silage, concentrate mixture, and whole diet is presented in Table 1. Grass silage and labeled grass silage were of high nutritional and fermentation quality as indicated by low concentrations of indigestible NDF, fermentation acids (81 and 68 g/kg of DM, respectively), and low proportion of AN in total N (80 and 57 g/kg of N, respectively). The diet was formulated to supply sufficient CP (0.155 of DM) to meet the microbial AN requirements but to avoid inefficient utilization of ^{15}N-labeled AN due to excessively high ruminal AN concentrations (the mean daily concentration was 5.3, SD 2.07 mmol/L). Data on DMI, milk production and rumen pool sizes of digesta and N fractions are shown in Table 2. Generally, the differences in animal variables were small when ^{15}N doses were given as AN or SN.

Experimental Procedures

Production of ^{15}N-Labeled Grass. Labeled AN was provided as $(NH_4)_2SO_4$ with 10% enrichment of ^{15}N/N (Isotec, Miamisburg, OH). Primary growth Timothy grass (*Phleum pratense*) was grown on clay soil on a 25 m^2 field plot that was fertilized with 1,100 g of labeled $(NH_4)_2SO_4$. In addition, 6, 50, and 50 g of N, P, and K, respectively, were supplied as commercial compound fertilizer. Grass was cut at DM concentration of 178 g/kg, wilted for 3 h at 20°C to DM concentration of 220 g/kg, chopped to a length of 3 to 5 cm, and subsequently ensiled in 9 laboratory scale silos (7.5 kg) using formic acid (5 mL/kg) as a preservative. Botanical analysis indicated that grass consisted mainly of *Phleum pratense* (0.96 of DM) of which 0.38 was at vegetative stage and 0.62 at head or early head stage. After 8 mo of ensiling, the silos were opened, the top surface layer was discarded, the contents from each silo were pooled, mixed, and a sub-sample was collected