

Rate of Release of Nitrogen From Alfalfa Hay Fractions into the Ammonia Nitrogen Pool *in vitro*

A. Melgar* and A. N. Hristov

Department of Animal and Veterinary Science, University of Idaho, Moscow, ID 83844-2330

This study compared the rate of bacterial breakdown of nitrogen (N) fractions from ^{15}N -labeled alfalfa hay in the rumen *in vitro*. Nitrogenous fractions from green house-grown alfalfa hay were isolated and incubated *in vitro* with mixed ruminal populations for eight hours. The following N fractions were prepared: Insoluble in water N (InsN), Soluble protein N (SolPN), Non-protein N (NPN), Neutral-detergent insoluble N (NDFN), and Neutral-detergent soluble N (NDFsolN). Data for NDFsolN were derived by the difference between InsN and NDFN. Casein labeled with ^{15}N was used as a control. Blank vessels without N source were also incubated. Incubation was repeated three times ($n=3$). SolPN was separated from NPN through precipitation with trichloroacetic acid. NDFN was prepared using standard NDF procedure. All N fractions were freeze-dried and incubated with ruminal inoculum/buffer media containing sugars, starch, and pectin as energy sources. Concentration of ^{15}N from alfalfa hay in the incubation media was 5.6 mg/L. Samples were taken at 0, 0.25, 0.5, 1, 2, 4, 6, and 8 h. Nitrogen-15 enrichment of ammonia N was determined through diffusion following precipitation of particulate and microbial mass and soluble proteins. Enrichment data were fitted to linear regression. Data were analyzed using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC).

Inclusion of NPN in the media resulted in an immediate release of isotope into the ammonia N pool (Figure 1). Compared to the other N fractions, alfalfa NPN had the largest ($P < 0.0001$) intercept (0.468 atom percent excess, APE). Of the other N fractions, SolPN and NDFsolN had greater ($P < 0.05$) intercepts than NDFN (0.048, 0.032, and -0.003 APE, respectively). The greatest ($P < 0.05$ to $P < 0.0001$) rate of release of ^{15}N into the ammonia N pool was associated with SolPN (0.061 APE/h). The rates of ^{15}N release from InsN and NPN (0.040 and 0.033 APE/h, respectively) were greater ($P < 0.05$) than from NDFN and ^{15}N -casein (0.012 and 0.016 APE/h, respectively).

This study suggested a very rapid microbial deamination of alfalfa hay NPN (amino acids and peptides) and soluble protein fractions in the rumen, which is in accordance with the relatively low by-pass of alfalfa protein N *in vivo*. The extent of release of casein N into the ammonia N pool was relatively low suggesting microbial utilization was taking place before deamination.

Figure 1. Release of ^{15}N from ^{15}N -labeled alfalfa hay N fractions into ruminal ammonia N *in vitro*

