Differential Ruminal Degradation of Alfalfa Proteins

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Alfalfa (*Medicago sativa*) has one of the highest crude protein contents among forage crops but the crude protein is rapidly and extensively degraded in the rumen. Even though research has demonstrated variability in alfalfa for degradation of crude protein, currently there are no known reports on the degradation of specific actual proteins in alfalfa. Our objectives were to i) identify the major specific actual proteins in alfalfa, and ii) quantify the rate of ruminal degradation of those major proteins after two hours digestion in the rumen.

Vernal and experimental alfalfa lines BC79, and Salt II were chosen for evaluation because of their distinct backgrounds. Plants were grown in a greenhouse with supplemental light. Single plants were harvested at 10% bloom and dried at 32ºC to simulate field conditions. Plant material was ground to 2 mm and samples of each were sealed in dacron bags and incubated in the rumen of a steer for 0, 45, and 120 min. Proteins from the single plants of the three alfalfa's were labeled with different fluorescent dyes for each of the time points and separated on single 2D gels. Gels were scanned for fluorescence representing quantities of the individual proteins from each of the time points.

Twenty six individual proteins were characterized, representing on average 36% of the total protein detected. Significant variation for rate of protein degradation was observed for germplasm source and for proteins after 45 and 120 min (P=0.003). The digestion rate for some proteins differed (P=0.05) among genotypes. Most proteins were uniformly digested across genotypes. After 45 min nine proteins averaged 75% or more remaining, 12 had 50% or less remaining, and six were intermediate. After 120 min four proteins averaged greater than 80% remaining, seven between 80 and 50%, and 16 less than 50% remaining. Generally proteins were uniformly more digested with increasing time and differences in disappearance became more pronounced with time. However, a few proteins showed little change with time. The rate of digestion differed for 2 and 10 proteins among genotypes after 45 and 120 m respectively. Several proteins were observed with large differences between means and associated large standard deviations but the genotypes were not statistically different. More replications and a lower error certainly would have improved separation. Overall ranking of genotypes did not change between 45 min to 120 min, Vernal was the least digested with BC79 and SaltII more digested and essentially the same. It was observed that between 45 and 120 min percent remaining protein for Vernal decreased about 10% while percent remaining protein for both BC79 and SaltII decreased about 15%. The proteins selected for characterization represented 40.7, 32.9, and 34.8 % of the total protein detected in Vernal, SaltII and BC79 respectively. Of the protein content characterized individual proteins as a percent of the total ranged from 41% (large subunit of RUBISCO) to 0.29 % (Malate dehydrogenase precursor). Total content of proteins that differed for digestion rate among genotypes ranged from 7 to 1%.

The results suggest variability among individual proteins and the potential to develop alfalfa with more protein that degrades slowly in the rumen and possibly escapes degradation in the rumen.