

*Cattlemen's Day 1996*

## IN VITRO ESTIMATION OF RUMINAL PROTEIN DEGRADABILITY OF FORAGES

I.E.O. Abdelgadir, R. C. Cochran,  
E. C. Titgemeyer, and E. S. Vanzant<sup>1</sup>

### Summary

Ruminal degradation of alfalfa and prairie hay protein was estimated using a proteolytic enzyme from *Streptomyces griseus* with or without pretreatment with cellulase or a broad spectrum carbohydrase (driselase). Estimates of the undegradable intake protein (UIP) as a percentage of total protein derived from the protease alone were higher than that measured in the animal (i.e., *in vivo*). Pretreatment of hay samples with cellulase (48 h incubation) or driselase improved the accuracy of UIP predictions compared with those determined using the protease alone.

(Key Words: Protein Degradability, Cellulase, Driselase, Forage.)

### Introduction

Forage protein includes that degraded in the rumen (degradable intake protein = DIP) and that escaping the rumen (undegradable intake protein = UIP). New feeding systems for ruminants require knowledge of the DIP and UIP contents of feedstuffs. Either of these can be measured by use of intestinally fistulated animals (*in vivo*), by nylon bags placed in the rumen of fistulated livestock (*in situ*), or by laboratory procedures. Once one fraction is known (DIP or UIP), the other can be calculated as the difference from total protein. *In vivo* and *in situ* estimations of UIP require maintenance of intestinally and (or) ruminally fistulated animals, which are typically unavailable to commercial feed laboratories. *In vitro* procedures using

semipurified proteolytic enzymes have shown promise as routine laboratory techniques for this purpose. However, fibrous components of forages may affect results derived from proteolytic enzymes alone. Therefore, we designed this experiment to evaluate the effect of pretreating forages with fiber digesting enzymes (cellulase or driselase) when attempting to determine the UIP value using a standard proteolytic enzyme assay.

### Experimental Procedures

Ruminal protein degradation of samples of alfalfa and prairie hay samples was estimated using a protease from *Streptomyces griseus*. The *in vitro* procedure was preceded by incubation with cellulase, driselase, acetate buffer, or no pretreatment. Cellulase and driselase concentrations were determined in a preliminary experiment and represented 8000 units of cellulase and 800 mg of driselase per g of hay sample. Cellulase, driselase, or acetate buffer pretreatments consisted of incubating alfalfa or prairie hay samples containing 15 mg nitrogen at 39°C for 2 or 48 hours. After the appropriate incubation time, samples were treated with the *S. griseus* protease for .25, .5, 1, 2, 4, 8, 12, 24, and 48 h. Following incubation, samples were filtered, residues were washed with deionized water, and nitrogen content in residues was determined. The UIP content was expressed as a percentage of total protein. In each run, cellulase, driselase, and acetate buffer pretreatment, no pretreatment (control), and blanks were included.

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<sup>1</sup>KSU Agricultural Research Center - Hays.

## Results and Discussion

The UIP contents determined *in vivo* for the alfalfa and prairie hay samples were 17 and 44% of total protein, respectively. Determined using the *S. griseus* procedure without pretreatment, the UIP content of alfalfa and prairie hay samples were 27 and 53% of total protein (Table 1). For both forages, the UIP content was overestimated by the *S. griseus* procedure when no pretreatment was used. For both alfalfa and prairie hay, carbohydrase pretreatments resulted in lower UIP estimates that were closer to the *in vivo* values than when no pretreatment was used.

Driselase generally yielded lower UIP estimates than cellulase. For alfalfa hay, the duration of pretreatments exerted only a slight effect on UIP estimates when driselase was used, whereas for prairie hay, the 48-hour cellulase and driselase pretreatments resulted in lower UIP values. UIP estimates with pretreatment were more similar to the *in vivo* values than those from hay without carbohydrase pretreatment. This improvement in UIP prediction was most evident when alfalfa hay was pretreated with driselase for 48 hours and when prairie hay was pretreated with driselase for 2 hours or cellulase for 48 hours.

**Table 1. Effect of Pretreatment on the Undegradable Intake Protein Content (% of Total Protein) of Alfalfa and Prairie Hay Determined by *Streptomyces griseus* Protease**

Forage	<i>In vivo</i>	Cellulase		Driselase		Acetate Buffer		No Pre-treatment	SEM
		2 h	48 h	2 h	48 h	2 h	48 h		
Alfalfa hay <sup>abcd</sup>	17	22.1	21.4	12.3	14.1	28.4	28.0	27.2	.52
Prairie hay <sup>abcde</sup>	44	61.5	45.6	43.8	37.8	55.7	58.1	53.1	1.14

<sup>a</sup>No pretreatment vs pretreatment ( $P < .05$ ).

<sup>b</sup>Cellulase vs driselase ( $P < .05$ ).

<sup>c</sup>Acetate buffer vs no pretreatment ( $P < .05$ ).

<sup>d</sup>Driselase treatment for 2 vs 48 h ( $P < .05$ ).

<sup>e</sup>Cellulase treatment for 2 vs 48 h ( $P < .05$ ).