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## Estimating Rumen Undegradable Protein in

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# Estimating Rumen Undegradable Protein in Smooth Bromegrass and Legumes

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Undegradable protein values for birdsfoot trefoil were higher than for alfalfa or kura clover.

## Summary

*An in situ trial was conducted to compare estimates of rumen undegradable protein (UIP) using a single incubation time point and rates of degradation. Four forage samples (three legumes and one grass) were incubated in situ for their mean retention time estimated from in vitro dry matter disappearance plus a 10-hour lag time as well as for a time point equal to 75% of the total mean retention time (mean retention time plus lag). The UIP values obtained from the fractional rates of degradation and passage were more highly correlated with those estimated from 75% of the total mean retention time ( $R^2 = 0.99$ ) than those estimated from the total mean retention time ( $R^2 = 0.62$ ). The UIP of birdsfoot trefoil was higher than that in the other forages.*

## Introduction

The standard method for estimating the potentially digestible fraction of protein that escapes rumen degradation uses a first-order disappearance model which assumes that ingested particles can pass out of the rumen immediately. Some particles may not escape out of the ru-

men for some time, however, and may undergo digestion during this time. Accounting for a lag in passage by adding 10 hours (suggested by previous research) to the mean retention time (MRT) represents the total MRT (TMRT) in which particles may be degraded. Neutral detergent insoluble nitrogen (NDIN) was used to directly estimate the UIP of forages in this experiment (Lamothe et al., this report).

Diet and clip samples previously were collected from smooth bromegrass pastures interseeded with birdsfoot trefoil, alfalfa, or kura clover (2002 Nebraska Beef Report, pp. 20-21). The legumes supplied fixed nitrogen for grass production and supplied additional protein for the yearlings grazing the forage. The UIP of the legumes is important because degradable protein is in excess of cattle needs and UIP is usually limiting for yearlings. The objective of this study was to compare UIP single incubation estimates obtained from forage samples at 75% TMRT and TMRT in addition to rates of NDIN degradation for three legumes and smooth bromegrass.

## Procedure

### Forage Samples

Four forage samples were included in the in situ trial: alfalfa, birdsfoot trefoil, kura clover and smooth bromegrass. The source of the forages were smooth bromegrass pastures interseeded with legumes at the Research and Development Center of the University of Nebraska, near Ithaca, Neb. There were two sample types for each forage: diet 23 and clip 1. Diet 23 samples were collected using four ruminally fistulated steers grazing the following: smooth

bromegrass (BROME), alfalfa and bromegrass (ALF), birdsfoot trefoil and bromegrass (BFT), or kura clover and bromegrass (KURA). Diet 23 samples are a composite of diet 2 and diet 3 samples and represent the midpoint of a grazing period (2002 Nebraska Beef Report, pp. 20-21). There were four periods (May through September) in which diet 23 forage samples were collected. The clip samples are from one collection period (May) and are composed of only the single forage: smooth bromegrass (cBROME), alfalfa (cALF), birdsfoot trefoil (cBFT), or kura clover (cKURA). Masticate (diet) and clip samples were freeze-dried and ground to pass through a 2-mm screen. A subsample was ground through a 1-mm screen for IVDMD analysis.

### In Situ Procedure

The experimental procedure used in this experiment was similar to that described by Lamothe (this report). Incubation time points included 10 hours, 75% TMRT, TMRT, and 96 hours and were estimated using the following equation:

$$\text{kp (\%/hour)} = 0.07 \text{ IVDMD (\%)} - 0.20$$

The inverse of the kp was used to determine the MRT, and a 10-hour lag time was added to the estimated MRT to yield the TMRT.

### Calculations

NDIN was measured on each in situ residue as well as on the original sample allowing for the construction of a degradation curve for NDIN. A first-order

**Table 1. Original CP of diet and clip samples, potentially digestible NDIP (% DM) remaining from 0 hour, 10 hour, 75% TMRT, and TMRT incubations, and the indigestible fraction (96 hour).**

Item	Original CP <sup>a</sup>	Incubation Time				96
		0 <sup>b</sup>	10 <sup>b</sup>	75% TMRT <sup>b</sup>	TMRT <sup>b</sup>	
Diet 23 <sup>c</sup>						
ALF	14.05	3.83	1.80	.56	.18	1.23
BFT	15.66	3.60	1.63	.60	.31	1.05
KURA	17.74	3.581	.21	.30	.34	.88
BROME	11.34	3.59	1.63	.54	.32	1.01
Clip 1 <sup>d</sup>						
cALF	13.40	2.50	.99	.34	.20	1.24
cBFT	15.03	2.74	1.19	.53	.37	1.48
cKURA	15.48	2.23	.67	.24	-.06	.64
cBROME	13.22	4.17	2.14	.66	.18	1.01

<sup>a</sup>Percentage of DM.

<sup>b</sup>96 hour values have been subtracted.

<sup>c</sup>Alfalfa and smooth brome grass (ALF), birdsfoot trefoil and smooth brome grass (BFT), kura clover and smooth brome grass (KURA), and smooth brome grass (BROME).

<sup>d</sup>Alfalfa (cALF), birdsfoot trefoil (cBFT), kura clover (cKURA), and smooth brome grass (cBROME).

**Table 2. Rate of degradation (%/hour) of NDIP of diet and clip samples from 0 to 10 hours, 10 hours to 75% TMRT, and 75% TMRT to TMRT.**

Item	0 - 10 <sup>ac</sup>	10 - 75% TMRT <sup>abc</sup>	75% TMRT - TMRT <sup>b</sup>
Diet 23 <sup>d</sup>			
ALF	7.72	8.24	10.08
BFT	8.40	7.98	8.23
KURA	11.53	15.73	3.35
BROME	7.59	8.26	9.36
Clip 1 <sup>e</sup>			
cALF	9.41	8.05	2.86
cBFT	8.85	8.21	3.61
cKURA	13.91	12.05	13.43
cBROME	6.70	9.44	5.52

<sup>a</sup>0 - 10 not different from 10 - 75% TMRT (P = 0.3253 and P = 0.8690) for Diet 23 and Clip 1, respectively.

<sup>b</sup>10 - 75% TMRT not different from 75% TMRT - TMRT (P = 0.2442 and P = 0.3027) for Diet 23 and Clip 1, respectively.

<sup>c</sup>Forage effect (P = 0.0202).

<sup>d</sup>Alfalfa and smooth brome grass (ALF), birdsfoot trefoil and smooth brome grass (BFT), kura clover and smooth brome grass (KURA), and smooth brome grass (BROME).

<sup>e</sup>Alfalfa (cALF), birdsfoot trefoil (cBFT), kura clover (cKURA), and smooth brome grass (cBROME).

**Table 3. Estimated UIP (% DM) of diet samples using three different approaches.**

Item	Equation <sup>a</sup>	75% TMRT <sup>bc</sup>	TMRT <sup>bc</sup>
Diet 23 <sup>df</sup>			
ALF	1.96	1.80	1.41
BFT	1.73	1.65	1.35
KURA	1.14	1.18	1.21
BROME	1.65	1.56	1.33
Clip 1 <sup>eg</sup>			
cALF	1.75	1.59	1.44
cBFT	2.14	2.01	1.84
cKURA	.84	.88	.58
cBROME1	.81	1.67	1.19

<sup>a</sup>UIP = pot dig NDIN \* [kp/(kp + kd)] + undig NDIN; corrected for passage lag time.

<sup>b</sup>In situ incubation at 75% TMRT and TMRT.

<sup>c</sup>75% TMRT UIP value different from TMRT UIP value for Diet 23 (P = 0.0009) and Clip 1 (P = 0.0105).

<sup>d</sup>Forage (P = 0.0007) and time (P < 0.0001) effect.

<sup>e</sup>Forage effect (P < 0.001).

<sup>f</sup>Alfalfa and smooth brome grass (ALF), birdsfoot trefoil and smooth brome grass (BFT), kura clover and smooth brome grass (KURA), and smooth brome grass (BROME).

<sup>g</sup>Alfalfa (cALF), birdsfoot trefoil (cBFT), kura clover (cKURA), and smooth brome grass (cBROME).

disappearance model was used to calculate the rates of ruminal degradation (kd) for each in situ CP fraction. The natural logarithm of the percentage of NDIN remaining (corrected for the 96-hour indigestible fraction) was regressed against time to calculate kd (slope of the regression line).

Data were analyzed using the MIXED procedure of SAS. Fixed effects in the model included: forage (alfalfa, birdsfoot trefoil, kura clover, and brome), time (period 1, period 2, period 3, and period 4), and incubation time (10 hour, 75% TMRT, and TMRT).

## Results

The initial, undegraded protein remaining, and indigestible fraction are shown in Table 1 for diet 23 and clip 1 samples. These values then were used to calculate rates of degradation and UIP values. There were no differences between rates of degradation for the three time periods—0 to 10 hours, 10 to 75% TMRT, and 75% TMRT to TMRT (Table 2). This was the case for both sample sets, clip samples and diet samples (diet 23). This suggests a constant rate of degradation for these forages from zero to TMRT.

Rates of degradation are shown in Table 2. There was a significant treatment x forage interaction (P = 0.0255) for diet 23 samples. From 10 to 75% TMRT (diet 23), the rate of degradation for KURA was significantly higher than ALF, BFT, or BROME (P < 0.05). The rates of degradation among forages from 0 to 10 hours or 75% TMRT to TMRT were not different for diet 23 samples (P > 0.05). Rates of degradation for clip samples were not different for the four forages (P > 0.05) with the exception of the rate from 0 to 10 hours for KURA being higher than BROME (P = 0.0421).

Values of UIP obtained from the competition of kp and kd represent mechanisms in the rumen and may be the most accurate estimates; therefore, the UIP values using kp and kd plus accounting for a lag were regressed linearly on the estimates from a single incubation time point, either 75% TMRT or TMRT. Table 3 shows the UIP values obtained

(Continued on next page)

for the diet and clip samples obtained from these three different approaches. There were two significant interactions for diet 23 samples: treatment (75% TMRT and TMRT)  $\times$  forage ( $P=0.0433$ ) and forage  $\times$  sampling month ( $P=0.0139$ ).

Estimates of UIP from 75% TMRT incubations were more highly correlated with those calculated from an equation using fractional rates of digestion and passage ( $R^2=0.99$ ) than estimates of UIP from TMRT incubations ( $R^2=0.62$ ). The relationship observed was consistent with Lamothe's single incubation UIP estimates for meadow and range

pastures ( $R^2=0.95$  and  $R^2=0.53$  for 75% TMRT and TMRT, respectively) when compared to the equation values for UIP.

The diet samples likely contain variable amounts of legume. Alfalfa, birdsfoot trefoil and kura clover pastures contained 40, 20 and 50 % legume, respectively. Therefore, the clip samples were evaluated to determine the protein degradability of the actual legumes. The UIP values for both the diet samples (legume and grass) as well as the clip samples (legume or grass) were consistent with the use of the equation or 75% TMRT (Table 3). The UIP values were

higher for the birdsfoot trefoil than for the alfalfa or kura clover ( $P<0.05$ ). Kura clover values were consistently low. The UIP values for birdsfoot trefoil may be higher than smooth brome grass, but the UIP may not be sufficiently high to increase the UIP content of the diet selected from the brome grass pasture interseeded with birdsfoot trefoil.

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<sup>1</sup>Heather Haugen, graduate student; Terry Klopfenstein, professor, Animal Science, Lincoln; Mark Ullerich, former graduate student; Casey Macken, Kimberly Whittet, and Tim Loy, research technicians.

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## Influence of Rinsing Technique and Sample Size on *In situ* Protein Degradation

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Use of machine rinsing or increasing sample size does not change *in situ* dry matter disappearance or undegradable intake protein values of soybean meal or Soypass.

### Summary

Four experiments were conducted to evaluate effects of *in situ* bag rinsing technique and sample size on the variation of undegradable intake protein (UIP) and dry matter disappearance (DMD) of soybean meal (SBM) and Soypass, a heat-treated soybean meal. Five rinsing techniques and five sample sizes were used to test effects. Soybean meal had higher DMD, lower UIP and higher variance for UIP than Soypass. A steer difference was noted for experi-

ments with steer as a replication and also contributed a larger effect than day and run within day. Rinsing technique and sample size were not significant in concentrate fed steers but were in mixed diet steers. There was a rinsing difference with highest machine rinses having higher DMD and lower UIP values. A size difference was noted with largest sample size having lowest DMD and highest UIP. No difference was found between hand and machine rinsing and no evidence was found to eliminate the use of an increased sample size.

### Introduction

Over the past twenty-five years, *in situ* digestion techniques have been used extensively for measuring ruminal degradation of feedstuffs. Moreover, *in situ* digestion techniques are commonly used to predict undegradable intake protein (UIP) value of protein sources. However, *in situ* techniques suffer from variation involving rinsing techniques and sample sizes. If incubated samples are washed too thoroughly, undigested

sample may be lost. If sample size is increased too much, dry matter disappearance (DMD) may be inhibited. Assays of rapidly degradable protein sources are influenced both by variation in DMD and UIP. Also, rapidly degradable feedstuffs incubated with small initial sample sizes leave minimal residue for further analysis. If the initial sample size can be increased, more residue will be remaining for subsequent analysis. Error across technicians may further contribute to the variation of *in situ* digestion techniques. Therefore, the objectives of this study were to evaluate the effect of *in situ* bag rinsing techniques and sample size on the variation of UIP and DMD of soybean meal (SBM) and Soypass, a heat-treated soybean meal.

### Procedure

Four experiments were conducted to evaluate effects of *in situ* bag rinsing technique and sample size on UIP and DMD of SBM and Soypass. All four experiments were conducted under similar conditions. Samples were weighed as