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# Estimating In Situ Degradability of Protein in Forages

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In situ neutral detergent fiber nitrogen is an effective method of estimating undegraded intake protein in forages. The information obtained allows for more accurate protein formulation of ruminant diets.

## Summary

*A method was developed for measuring undegraded intake protein (UIP) in forages. Neutral detergent fiber nitrogen (NDFN) was assumed to be the potential ruminally-undegradable fraction. In situ incubations were completed on eight forages to determine rates of digestion and rates of passage were used to calculate UIP. When compared to in situ UIP values determined by both uncorrected and microbial-corrected nitrogen, values found using NDFN were not different from the purine method and were more precise. Furthermore, NDFN gave values for four of the samples that were highly correlated to in vivo values determined for those forages.*

## Introduction

Current applications of beef cattle nutrition such as the newly-revised National Research Council's nutrient requirements of beef cattle use a metabolizable protein system to calculate animal requirements because the protein needs of ruminants are met by both microbial protein and undegraded intake protein (UIP). A metabolizable

protein system describes the total amount of protein absorbed by the small intestine from these two sources and is superior to expressing requirements only as crude protein in the diet.

To take advantage of such a system, accurate information about the degradability of protein in the diet is required. Degradability information is used to calculate the amount of UIP that contributes to the metabolizable protein pool.

Many methods currently exist for measuring protein degradability of feedstuffs. The in vivo method is accepted as the standard because it provides an actual UIP value for the feedstuff. Animals are fed the diet in question and digesta samples are obtained. Laboratory analyses are conducted to measure what proportion of the total protein reaching the small intestine is UIP.

However, there are many disadvantages to the in vivo method. Animals with the ruminal and intestinal fistulas are needed. Flow rate and microbial markers are used to calculate what proportion of the metabolizable protein pool originates from the diet, microbes, or the animal itself. These markers add considerably to the time and expense required to complete this measurement and may be inaccurate. Therefore this method is not practical as it is neither inexpensive nor simple for a commercial laboratory to perform.

Attempts have been made to develop a simple laboratory method that could measure feed protein degradability. Commercially-produced enzymes have been tested and some success has been reported. Such methods are simple, rapid, and do not require the use of an animal. However, degradability estimates obtained using

commercial enzymes may not correlate well with the accepted in vivo estimates.

Another method used is the in situ dacron bag. Samples are incubated in a ruminally-fistulated animal and the amount of UIP can be determined. However, different estimates of degradability may be obtained from this method depending on whether or not attached microbial protein is measured. While the use of such microbial markers as purines is a standard practice, such methods are labor intensive.

Previous researchers stated that feed protein that is insoluble in neutral detergent solution makes up the potential UIP fraction and is partially digestible in the rumen.

The objective of this experiment was to determine if neutral detergent fiber nitrogen (NDFN) of forages incubated in situ was an effective estimate of UIP when compared to in situ values (both uncorrected for microbial protein and corrected with purines) and in vivo values for those forages.

## Procedure

The standardized method for in situ incubation was used. Five grams of sample were placed in dacron bags and those bags were placed into several mesh bags. These mesh bags were placed into the rumen for incubation. The mesh bags were then washed thoroughly in warm water and the bags and residue were then dried.

Samples tested included two alfalfa hays, two Sandhills meadow hays, one brome hay, one prairie hay, and two range samples. They were incubated in a ruminally-fistulated steer that was fed brome hay containing 8 percent CP

**Table 1. Undegraded intake protein values (% DM).**

Sample	CP	UNCORR <sup>a</sup>	PUR	NDFN
Brome hay	14.4	5.04	3.54	3.57
Prairie hay	6.8	3.77	3.14	3.14
Alfalfa hay #1	20.3	4.02	3.19	2.87
Alfalfa hay #2	30.0	5.34	4.25	3.65
Meadow hay #1	16.2	3.54	2.20	2.28
Meadow hay #2	7.7	2.45	1.38	1.68
Range diet #1	12.0	4.87	3.50	3.15
Range diet #2	5.6	1.74	0.84	1.24
Mean UIP	—	3.84 <sup>b</sup>	2.75 <sup>c</sup>	2.70 <sup>c</sup>
SE <sup>d</sup>	—	0.14	0.10	0.07

<sup>a</sup> UNCORR uses total in situ N to calculate UIP.

PUR uses total in situ N corrected for microbial N.

NDFN uses in situ N that is insoluble in neutral detergent.

<sup>b,c</sup> Means with unlike superscripts differ ( $P < .05$ ).

<sup>d</sup> Standard error for each method.

**Table 2. Correlation coefficients of methods.**

METHOD <sup>a</sup>	UNCORR	PUR	NDFN	IN VIVO
UNCORR	—	.821	.812	.685
PUR	.821	—	.921	.893
NDFN	.812	.921	—	.954
IN VIVO	.685	.893	.954	—

<sup>a</sup> UNCORR uses total in situ N to calculate UIP.

PUR uses total in situ N corrected for microbial N.

NDFN uses in situ N that is insoluble in neutral detergent.

IN VIVO is considered the standard UIP value for a forage.

(DM basis). The five vegetative samples were incubated for 4, 10, and 16 hours and the dormant samples were incubated for 8, 16, and 24 hours. Incubations were replicated three times on consecutive days.

The residue in each bag was analyzed for nitrogen, purine, and NDFN. A separate experiment was conducted to determine the purine to nitrogen ratio for our experimental protocol. In situ residue was analyzed for purine and

nitrogen content before and after the NDF procedure.

Rates of digestion (Kd) for potential UIP were calculated using residual nitrogen alone (UNCORR), residual nitrogen corrected for microbial nitrogen as determined by the purine method (PUR), and NDFN. Rates of passage (Kp) of 5%/hour for vegetative samples and 2%/hour for dormant samples were used. The potential UIP pool for each method was calculated using the y-

intercept of the rate of digestion equation.

The following equation was used to calculate UIP on a dry matter basis:

$$\text{UIP} = \frac{\text{Kp}}{\text{Kp} + \text{Kd}} * \text{potential UIP pool} * 6.25$$

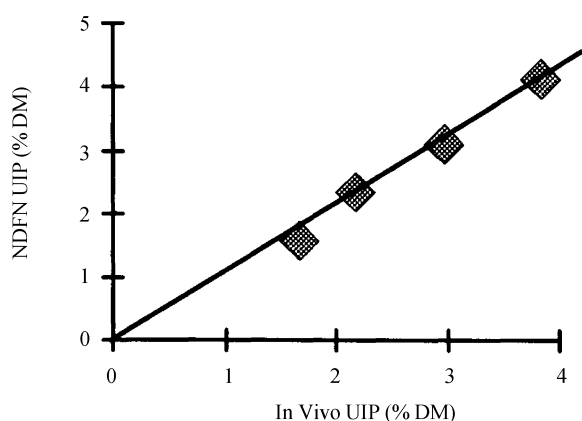
## Results

The UIP values for UNCORR were higher than either PUR or NDFN ( $P < .05$ , Table 1). When the purine to nitrogen ratio determined herein (.14) was applied, PUR was not different than NDFN ( $P > .05$ ). The standard error for mean NDFN was lowest, indicating that it is the most precise method. These results support our hypothesis that NDFN is equal to or more accurate than PUR, which is currently an accepted method for correcting in situ residue for microbial nitrogen. Additionally, the necessity of an accurate purine to nitrogen ratio when estimating PUR UIP illustrates one of the disadvantages of that method.

A correlation analysis was conducted to compare combinations of the four UIP methods (Table 2). NDFN and PUR were highly correlated ( $r = .921$ ), showing that the two procedures ranked the samples similarly.

In vivo UIP values for four of the samples were correlated with each laboratory procedure (Table 2). Individual NDFN values were ranked similarly in respect to in vivo values (Figure 1). NDFN yielded the highest correlation coefficient with in vivo values of all the in situ methods ( $r = .954$ ), indicating that it is the most accurate laboratory procedure.

In summary, in situ NDFN is an accurate and precise way to measure UIP in forages when compared to either not correcting for microbial nitrogen or using the purine method as a correction. NDFN eliminates the need for a purine to nitrogen ratio and is simpler to perform than PUR. However, it does require a ruminally-fistulated animal.

**Figure 1. Correlation between NDFN and In vivo.**

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