Kansas Agricultural Experiment Station Research Reports

Volume 0
Issue 1 *Cattleman's Day (1993-2014)*

Article 541

1997

Estimating the undegradable intake protein content of two forages by different commercial proteases

I.E.O. Abdelgadir

R.C. Cochran

E.S. Vanzant

Evan C. Titgemeyer

Follow this and additional works at: https://newprairiepress.org/kaesrr

 Part of the Other Animal Sciences Commons

Recommended Citation

Abdelgadir, I.E.O.; Cochran, R.C.; Vanzant, E.S.; and Titgemeyer, Evan C. (1997) "Estimating the undegradable intake protein content of two forages by different commercial proteases," *Kansas Agricultural Experiment Station Research Reports*: Vol. 0: Iss. 1. https://doi.org/10.4148/2378-5977.1944

This report is brought to you for free and open access by New Prairie Press. It has been accepted for inclusion in Kansas Agricultural Experiment Station Research Reports by an authorized administrator of New Prairie Press. Copyright 1997 Kansas State University Agricultural Experiment Station and Cooperative Extension Service. Contents of this publication may be freely reproduced for educational purposes. All other rights reserved. Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. K-State Research and Extension is an equal opportunity provider and employer.

K-STATE Research and Extension

Estimating the undegradable intake protein content of two forages by different commercial proteases

Abstract
We evaluated the potential of several commercially available proteases for use in predicting the undegradable intake protein (UIP) concentrations o f alfalfa and prairie hay. Protease s differed in their estimates o f the rate of forage protein breakdown an d the amounts of different forage protein fractions . At least one protease appeared to yield acceptable predictions of UIP via a short-term, single time-point assay. Assays of this type deserve further consideration for commercial application.

Keywords
Cattlemen's Day, 1997; Kansas Agricultural Experiment Station contribution; no. 97-309-S; Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 783; Beef; Protein degradability; Proteases; Forages

Creative Commons License

This work is licensed under a Creative Commons Attribution 4.0 License.

This Research Report article is available in Kansas Agricultural Experiment Station Research Reports: https://newprairiepress.org/kaesrr/vol0/iss1/541

ESTIMATING THE UNDEGRADABLE INTAKE PROTEIN CONTENT OF TWO FORAGES BY DIFFERENT COMMERCIAL PROTEASES

I.E.O. Abdelgadir, R. C. Cochran
E. C. Titgemeyer, and E. S. Vanzant[1]

Summary

We evaluated the potential of several commercially available proteases for use in predicting the undegradable intake protein (UIP) concentrations of alfalfa and prairie hay. Proteases differed in their estimates of the rate of forage protein breakdown and the amounts of different forage protein fractions. At least one protease appeared to yield acceptable predictions of UIP via a short-term, single time-point assay. Assays of this type deserve further consideration for commercial application.

(Key Words: Protein Degradability, Proteases, Forages.)

Introduction

Current feeding systems for ruminants require knowledge of the proportion of forage protein degraded in the rumen (degradable intake protein = DIP) versus that escaping the rumen (undegradable intake protein = UIP). Measuring the DIP or UIP content using animals (i.e., via *in vivo* or *in situ* techniques) requires maintenance of intestinally or ruminally fistulated animals, which are expensive, require special care, and are frequently unavailable in commercial laboratory settings.

In vitro procedures using semipurified proteolytic enzymes have shown promise as routine laboratory techniques for estimating UIP, but in most cases, only concentrates and protein supplements have been tested extensively. Information about how these proteases work with forages is needed. Therefore, our objectives were to evaluate the potential of several commercial proteases for determining protein degradability, size of protein fractions, and the UIP content of forages. Values obtained using the proteases were compared with those obtained by *in situ* and *in vivo* methods in a previous experiment.

Experimental Procedures

Experiment 1. Four commercially available proteases were used to measure protein degradability in alfalfa and prairie hay. The proteases were from *Streptomyces griseus* (SGP), *Aspergillus oryzae* (AOP), *Ficus glabrata* (ficin), or bromelain from pineapple stem (BR).

For the SGP procedure, hay samples containing 14 mg N (.52 g of alfalfa or 1.64 g of prairie hay, air-dry basis) were incubated for 1 hour at 39°C in 40 ml of borate-phosphate buffer (pH 8.0). For the AOP, BR, and ficin procedures, .5 ml of triton X-100 and 20 ml of 1:1 mixture of *in vitro* rumen buffer (pH 6.8) and macromineral solution were added to hay samples. One ml of sodium azide (1% w/v) was added to all flasks as an antimicrobial agent. Following the 1-hour buffer incubation, 10 ml of SGP at .33 units/ml, AOP at 3.5 units/ml, BR at 5.0 units/ml, or ficin at 2.15 units/ml were added, and samples were incubated for .25, .5, 1, 2, 4, 8, 12, 24, and 48 hours. The 0-hour incubations were those subjected only to the 1-hour buffer incubation.

[1]KSU Agricultural Research Center - Hays.

Following exposure to the proteases, samples were filtered, residues were washed with 400 ml of deionized water, and nitrogen (N) contents of the residues were measured. Fractions and rates obtained were used to calculate the UIP contents of the forages using passage rates measured in a previous *in vivo* trial (average for both forages was approximately 2.9%/hour).

Experiment 2. Alfalfa and prairie hay samples were incubated at 39°C for 1 hour in an appropriate buffer solution, followed by addition of 10 ml of SGP solution containing .33, 3.3, or 33 units/ml; BR solution containing .5, 5.0, or 50 units/ml; or ficin solution containing .215, 2.15, or 215 units/ml. Based on results observed in Exp. 1, the AOP enzyme was not used in Exp. 2. Samples were incubated for 2, 4, or 48 hours. Residual N was considered to represent the UIP content and was expressed as a percentage of total protein.

Results and Discussion

Experiment 1. The size of forage protein fractions and degradation rates (Table 1) estimated with different proteases were similar in some instances to those obtained by a standard *in situ* procedure. However, none replicated *in situ* methods consistently. These results agreed with other reports indicating lack of consistency between *in situ* methods and those based on protease enzymes. In contrast, combining degradation rates and fractions to estimate the UIP content yielded UIP estimates that, for the SGP, BR, and ficin proteases, were similar to those determined in animals (*in vivo*).

The UIP estimates from the AOP enzyme were significantly larger than those from the other enzymes, as well as those from the *in situ* and *in vivo* methods. We also observed that in several cases, the amount of N remaining after incubation in SGP, ficin, or BR for a defined length of time closely approximated *in vivo* UIP. As a result, we felt that further exploration of simple, single time-point assays was justified (see Exp. 2).

Experiment 2. The main focus of this experiment was to develop a rapid, commercially viable, UIP assay. We used a range of enzyme concentrations and incubation times to see if assay length could be reduced by using higher enzyme concentrations. The highest concentrations of ficin (21.5 units/ml) and BR (50 units/ml) resulted in viscous solutions, causing filtration problems that prevented adequate washing of the residue from solubilized N. Consequently, results obtained at these high enzyme concentrations, particularly at short incubation times, were unreliable.

The two combinations of enzyme concentration and incubation time that compared best to *in vivo* values were the 4-hour incubation in SGP at 33 units/ml and the 48-hour incubation in SGP at .33 units/ml (Table 2). Results with the long incubation, low concentration study concur with research from Cornell University. Although short-term incubations in ficin did not yield particularly good predictions of UIP across both forages, the 48-hour incubation at 2.15 units/ml yielded values reasonably close to *in vivo* values. The BR method yielded reasonable values in some cases for alfalfa but not for prairie hay.

In conclusion, single time-point estimates of UIP using SGP and possibly ficin appear to have potential for estimating forage UIP content in a commercial setting. The potential for short-term, single time-point assays of forage UIP across a wide array of forages and different stages of maturity deserves further evaluation.

Table 1. Nitrogen Pool Sizes and Degradability of Alfalfa and Prairie Hay Estimated by Commercial Proteases [a] (Experiment 1)

Item	*In situ*	SGP	AOP	Ficin	BR	SEM[b]
Alfalfa hay						
N fractions, % of total N [c]						
A	44.8	31.6	30.9	30.2	33.1	.23
B	50.4	45.1	24.6	52.7	51.5	1.08
C	4.8	23.3	44.5	17.1	15.4	1.09
UIP[d], % of crude protein	12.8	30.6	53.2	17.7	16.7	.23
Kd, hour^{-1}	.16	.16	.05	2.57	1.12	.24
Prairie hay						
N fractions, % of total N						
A	32.7	24.4	21.1	22.7	20.6	.43
B	45.9	25.4	26.1	24.7	24.1	.38
C	21.4	50.2	52.8	52.6	55.3	.17
UIP[e], % of crude protein	42.8	54.8	63.7	53.4	56.3	.15
Kd, hour^{-1}	.04	.15	.04	.74	.65	.04

[a]SGP = *Streptomyces griseus* protease; AOP = *Aspergillus oryzae* protease; BR = bromelain.
[b]SEM for protease treatments.
[c]B and C fractions estimated using a single-pool kinetic model where B = insoluble potentially degradable protein fraction and C = undegradable protein fraction; A = (100% - C - B); undegradable intake protein(UIP) = $B \times [K_p/(K_d + K_p)] + C$ where K_p = rate of passage (.029 hour^{-1}) and K_d = degradation rate of the B fraction.
[d]*In vivo* UIP = 16.6 ± 4.3, % of total protein.
[e]*In vivo* UIP = 44.5 ± 3.5, % of total protein.

Table 2. Effect of Protease Type, Concentration (unite/ml), and Incubation Time on UIP[a] Estimates for Alfalfa and Prairie Hay (Experiment 2)

	Streptomyces griseus								
Item	.33	3.3	33	.215	2.15	21.5[b]	.5	5	50[b]
	---------- UIP[a] estimate, % of total crude protein ----------								
Alfalfa hay[c]									
Incubation time, hour									
2	64.0	41.7	26.6	42.4	23.8	26.6	44.9	27.78	23.6
4	57.1	32.4	18.6	34.9	20.4	30.2	34.9	22.1	21.7
48	23.2	12.8	10.4	18.4	13.9	26.8	18.5	11.9	17.8
Prairie hay[d]									
Incubation time, hour									
2	70.9	55.9	51.6	68.6	60.8	81.5	76.0	66.7	79.1
4	67.6	53.4	47.7	66.5	58.9	81.3	72.0	63.5	73.2
48	50.6	38.9	30.7	53.8	44.8	57.9	63.0	55.0	59.1

[a]UIP = undegradable intake protein.
[b]Higher enzyme concentrations caused filtration difficulties resulting in unreliable estimates.
[c]*In vivo* UIP, % of total protein = 16.6 ± 4.3. SEM for protease UIP estimates = .81, LSD (P = .05) = 2.28. Within assay CV = 3.42% for first run and 4.04% for second; between assay CV = 6.87%.
[d]*In vivo* UIP, % of total protein= 44.5 ± 3.5. SEM for protease UIP estimates = .93, LSD (P = .05) = 2.627. Within assay CV = 1.48% for first run and 1.77% for second run; between assay CV = 2.82%.