Digestibility and Estimation of Undegradable Intake Protein (UIP) of Alfalfa, Bermuda Grass and Prairie Hay Using Different Methods

R.B. Basurto, H.T. Purvis II, T. Bodine, and G.W. Horn

Story in Brief

To estimate forage digestibility and undegradable intake protein (UIP) of alfalfa (ALF), bermuda (BER) or prairie hay (PRA), six crossed Angus steers were fed ad libitum in a replicated 3 x 3 Latin square design with 21-d experimental periods. Apparent digestibility of forage was estimated using the total fecal output method. The methods to estimate UIP were: 1) *in vivo* method, using ratio of purine:N of ruminal bacteria and purine concentration in duodenal digesta flow (*in vivo*), 2) laboratory method using a protease (*S. griseus*; STREP), and 3) fiber bound nitrogen, using the relation: NDIN * kp / (kp + kd), where kd is *in situ* digestion rate, kp is passage rate and NDIN is neutral detergent insoluble nitrogen, estimated as intercept of digestion kinetic regression of NDIN, (NDIN-1) or direct value of NDIN obtained in the lab (NDIN-2). Forage DM digestibility was greatest for ALF (61.9%), follow by BER (54.3%) and was lowest for PRA (45.1%). *In vivo* values for UIP % DM were 7.3, 3.0 and 1.5 for ALF, BER and PRA, respectively. All methods were similar in predicting the UIP of PRA, however they differed in predicting the UIP for ALF and BER. When the initial NDIN was considered as in NDIN-2, values for UIP were similar to STREP values. Overall, there seems to be bias in fiber bound nitrogen and enzymatic procedures when compared with *in vivo* protein estimation.

Key Words: Forage, Undegradable Intake Protein, *In vivo*, Methods, Digestibility.

Introduction

In general, dietary protein can follow two routes in rumen. First route is rumen microorganisms degrade dietary protein and use products for their growth and in the second, dietary protein escapes from rumen without being attacked. When animals consume only one forage species, their dietary protein consists of a mixture of proteins with different chemical characteristics and degree of susceptibility to ruminal degradation. Sniffen (1992) proposed that neutral detergent insoluble nitrogen, NDIN or fraction B3, is the main source of undegradable intake protein (UIP) in forages. Mass et al. (1999) concluded that in situ NDFN is an accurate method to estimated UIP. However, it is known that ruminal escape of proteins becomes larger as feed intake, which is related positively to passage rate, increases. Thus, a change in the retention time, or a change in passage rate, causes protein degradation to be altered (Schadt et al., 1999). Mertens (1987) developed the relation between rates of passage (kp) and digestion (kd) which determines the proportion of a protein that leaves rumen without being attacked. This proportion that escapes can be an estimated of UIP. Because *in situ* techniques are easily performed, a large amount of information on digestion rate exists in the literature. In contrast, because passage rates require more laboratory and animal work, the kp values in the literature are limited. This has stimulated the development of laboratory techniques to estimate the extent of protein degradation in rumen. So the objective of this trial was to compare different methods to estimate the original pool of UIP in three forages, alfalfa (ALF), bermuda grass (BER) and, prairie hay (PRA).

Materials and Methods

Six steers (491 kg) with ruminal and duodenal cannulae were used in a replicated 3 x 3 Latin square design. During each experimental period (21 d), the steers were adapted to one of three forages (alfalfa, bermuda grass and prairie hay; Table 1) for 11 d before beginning collection of samples. Any additional energy, protein or mineral was supplemented during the experiment. From 12 d to 16 d, the apparent digestibility was determined. *In situ* digestion rate was performed from 17 d to 21 d. To determine passage rate, the ruminal contents of animals were removed, weighed and sampled before (0800) and after feeding (1200) at 21 d. Steers were weighed at the beginning and the end of each experimental period.

Forages were chopped through a 3-cm screen and fed ad libitum. Forage and orts were weighed daily and returned to feeders. The total fecal output was collected daily, weighed and sampled for 4 d. The samples were dried at 50ºC for 96 h in an air-forced oven. Dried fecal samples of each animal were ground through a 2-mm screen in a Willey mill, and composed by weight for each period.

The methods to estimate the undegradable intake protein (UIP) were: 1) *in vivo* method, 2) laboratory method, using a protease enzyme, and 3) mathematic method.

In vivo Method). The ratio of purine:N of ruminal bacteria obtained from whole ruminal samples and nitrogen and purine concentration in duodenal digesta flow measured by using Cr_2O_3 and NH_3-N concentration in reconstituted duodenal samples were used to calculate UIP as % DM=(total nitrogen flow –(microbial nitrogen flow + NH_3-N flow) divided dry matter intake.

Laboratory Method. The UIP values were calculated as 100 minus degradable intake protein percentage, which was determined by measuring nitrogen disappearance during 48-h incubation in a solution containing the proteolytic enzyme from *Streptomyces griseus* (STREP; Roe et al., 1990).

Fiber Bound Nitrogen. The UIP was calculated using the relation: NDIN * kp / (kp + kd), where kp is the *in situ* digestion rate, kp is the passage rate and NDIN is the initial pool of neutral detergent insoluble nitrogen. To determined kd, eight nylon bags (10 x 20 cm and 53 μm pore size) with 5 g of forage were located in the rumen before morning feeding at 17 d. The bags were soaked in warm water (37º C) before their insertion into the rumen. The bags were removed after 2, 12 and 96 h of incubation. After incubation, the bags were lightly washed and then kept frozen until the end of the trial to complete this step in a washing machine. The neutral detergent insoluble nitrogen (NDIN) analysis was performed on *in situ* residue the percentage of NDIN in dry matter was transformed to its logarithmic expression and regressed against time (Bodine et al., 1999). The slope and intercept of regression were considered to be digestion rate (kd) and original pool of NDIN (NDIN-1), respectively. The direct determination of NDIN in forage (Table 1) was considered as a second estimate of original pool of NDIN (NDIN-2). To measure the kp, at d 21, the total rumen contents of each animal were removed and weighed before feeding (0800) and 4 h post-feeding (1200). The contents were mixed by hand, and then sampled. The samples (1 kg) were weighed and dried at 50ºC for 96 h in an air-forced oven. The remaining contents were returned to rumen. The kp was estimated as kp= consumption of

insoluble ash in acid detergent solution (ADIA) per hour divided by average amount of ADIA in rumen contents (Waldo et al., 1972).

The DIP values were calculated as: 100* ((crude protein % DM in forage – UIP % DM)/ crude protein % DM in forage).

Ground samples of forages, orts, feces, *in situ* residue, and rumen contents were analyzed for dry and organic matter by drying either at 100ºC overnight or ashing at 500ºC for 6 h. Nitrogen content of forages, feces, *in situ* residue, and NDIN were determined by Kjeldahl method. The fiber fractions of forages and *in situ* residue, and ADIA in forages and rumen content were analyzed by the methods proposed by Van Soest et al. (1991). Response variables were analyzed as a replicated 3 x 3 Latin square experimental design using GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model included square, period, animal and treatment (forages). The means were separated using least significant difference.

Results and Discussion

The chemical analysis of experimental forages is shown in Table 1. The animals fed ALF had greater forage intake (P<.05) on dry and organic matter basis than those fed BER or PRA (Table 2). Van Soest (1994) suggested that as NDF decreases, forage intake typically increases. In contrast, although BER and PRA had a similar NDF content, the forage intake was larger for BER than that for PRA. The lower intake of animals consuming PRA can be explained by a higher content of acid detergent fiber and a deficiency of ammonia in rumen.

Table 1. Chemical composition and fiber fractions of Alfalfa, Bermuda and Prairie hay on DM basis

Analysis	ALF	BER	PRA
	DM basis		
N (x6.25)	20.74	11.48	4.78
NDF[2]	54.02	70.32	70.43
ADF[2]	39.18	31.57	42.59

[1]ALF=alfalfa, BER=bermuda and PRA=prairie hay
[2]CP=crude protein, NDF=neutral detergent fiber; ADF=acid detergent fiber

As it was expected, ALF had the greatest digestibility (P<.05) and the lowest digestibility was for PRA. Despite of difference in NDF content, ALF and BER had similar passage rates (kp), which were higher than that observed for PRA (P<.05; Table 3). West et al. (1997) suggested that higher digestibility and digestion rate of bermuda NDF could increase the passage rate of bermuda. On the other hand, Freeman et al. (1992) reported that animals fed PRA without protein supplement had a kp of 2.3% h^{-1}, which is similar to observed value for PRA in the present study.

The digestion rate (kd) of NDIN was much lower (P<.05) in PRA than ALF and BER. Although BER had a kd that was slightly faster than that for ALF, no difference could be detected statistically.

Table 2. Least squares means of forage intake and apparent digestibility in steers fed alfalfa, bermuda

or prairie hay				
Intake	ALF	BER	PRA	SE
Dry matter, kg d^{-1}	13.23[a]	11.05[b]	7.63[c]	.42
Organic matter, kg d^{-1}	12.07[a]	10.45[b]	7.00[c]	.39
Digestibility				
Dry matter, %	61.94[a]	53.72[b]	45.12[c]	1.49
Organic matter, %	61.37[a]	54.33[b]	46.03[c]	1.42
Crude Protein, %	69.82[a]	56.89[b]	20.18[c]	3.00

[1]ALF=Alfalfa, BER=Bermuda and PRA=Prairie hay
[a,b,c]Means within the same row without common subscripts are different P<.05.

Table 3. Least squares means of particle passage and digestion rates of NDIN in animals fed alfalfa, bermuda or prairie hay

Variable	ALF	BER	PRA	SE
Particle passage, % $h^{-1,1}$	.035[a]	.031[ab]	.023[b]	.0023
Digestion rate % $h^{-1,2}$	.012[a]	.015[a]	.008[b]	.001

[1]ALF=alfalfa, BER=bermuda and PRA=prairie hay
[a,b,c]Means within the same row without common subscripts are different P<.05.
[2]From ruminal evacuation at 0 and 4 h post-feeding and ADIA as indigestible marker
[3]For *in situ* NDIN residue at 2, 12 and 96 h of incubation in rumen

Table 4 is a comparative table of UIP and DIP estimations using different methods. ALF had higher *in vivo* value (7.3%) for UIP than those for BER (3.0%) or PRA (1.5%) when compared on a % DM basis. All methods were similar in predicting the UIP of prairie hay, however they differed in predicting the UIP for alfalfa and bermuda. Although STREP has been considered to be an adequate method (Boderick, 1994), it gave higher values for BER and PRA than those observed *in vivo* when compared on a % DM basis. The method NDIN-1 ranked UIP for ALF and BER to be similar to each other yet higher than that for PRA. When lab value for NDIN (NDIN-2 method) was used, the method ranked UIP as the *in vivo* method did, but values for BER and PRA were slightly higher than those obtained *in vivo*. Expressed as degraded intake protein as percentage of total crude protein, the ALF, BER and PRA had similar *in vivo* values. Similar data over a range of nutritive forage values (Basurto et al., 2000) found that DIP, expressed as a percentage of CP, remains relatively constant throughout the calendar year using the STREP method. The DIP values for ALF and PRA obtained by other laboratory methods were similar to *in vivo* values (83.5 % and 55.5 % for ALF and PRA) reported by Vanzant et al. (1996). Overall forages UIP content of forage vary with method used when compared with *in vivo* estimation.

Table 4. Estimated percentage of undegraded (UIP) and degraded (DIP) intake protein of alfalfa, bermuda or prairie hay

	UIP, % DM				DIP, % of total CP			
Method	ALF[1]	BER	PRA	SE	ALF	BER	PRA	SE
In vivo	7.3[a]	3.0[b]	1.5[b]	0.53	65.0	74.0	67.4	6.70

STREP, %[2]	4.6	4.9	2.1	NA[4]	77.9	57.6	54.8	NA[4]
NDIN-1, %[3]	2.8[a]	2.7[a]	2.1[b]	.14	86.7[d]	76.4[e]	56.4[f]	1.26
NDIN-2, %[3]	4.7[a]	3.8[b]	2.1[c]	.11	77.1[d]	67.1[e]	54.7[f]	1.28

[1]ALF=alfalfa, BER=bermuda and PRA=prairie hay
[2]*Streptomyces griseus* protease method (Roe et al., 1990)
[3]Undegradable intake protein (UIP)=NDIN * kp/(kp+Kd); where NDIN was considered to be equal to the intercept of regression equation of NDIN digestion (NDIN-1) or direct determination of NDIN in the forages (NDIN-2)
[4]Not available
[a,b,c,d,e,f]Means within the same row without common subscripts are different P<.05.

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