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Nutrition

A Comparison of Techniques for Estimating Forage Digestion

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Summary and Implications

The objective of this study was to compare in situ and in vitro methods for determining rate and extent of forage digestion in the rumen of beef cattle. Duplicate polyester (10 × 20 mm) or ANKOM F57 filter bags containing 5.0 or 0.5 g of ground (2mm) bromegrass hay, respectively, were incubated in situ or in vitro (ANKOM Daisy^{II} Incubator) for 3, 6, 9, 12, 15, 18, 24, 36, and 96 hours. Bags were incubated in the rumen of 3 ruminally cannulated Angus × Gelbvieh cattle consuming bromegrass hay, or in rumen fluid collected from these cattle. At each time point, duplicate bags were removed, rinsed with tap water and dried (55°C). Residues were analyzed for OM, NDF, and CP. Estimates of OM digestibility were higher ($P \leq 0.03$) for in situ and in vitro at 15, 24, 36, and 96 hours. At incubation times of 6 through 24 hours, NDF digestibility was comparable ($P = 0.10$ to 0.44) between techniques, but differed ($P \leq 0.03$) at 3, 36, 48, and 96 hours of incubation. Forage CP digestibility determined in situ was greater ($P \leq 0.01$) than in vitro at all incubation times. Estimates of N fractions A, B, and C did not differ ($P \geq 0.12$) for either technique. However, estimated N degradation rate was eight fold higher ($P = 0.01$) for in situ (8.1%/hour) than in vitro (1.6%/hour), resulting in greater ($P = 0.0001$) estimated ruminally degradable protein of the forage for the in situ (73.6%) compared to the in vitro (53.8%) technique. Because estimates of forage digestibility differed depending on the technique employed, estimates of in situ or in vitro forage digestibility cannot be used interchangeably.

Key Words: In Situ, In Vitro, Digestibility

Introduction

As ruminant diet formulation has become more advanced, the need for more in depth knowledge of ruminal digestibility of certain feedstuffs is required. Although in vivo digestibility estimates are generally considered the most accurate, they are often very labor intensive and expensive to determine (Broderick, 1994), and thus in vitro or in situ methods can be used in lieu of in vivo studies to

determine ruminal digestibility. Notwithstanding, each technique has advantages over the other. The in vitro procedure is convenient and allows for fermentation end products to be collected (Varel and Kreikemeier, 1995) whereas in situ estimates allow for influences of the rumen environment (Uden and Van Soest, 1984). However, little research has been conducted to determine differences in extent of digestion over time between these techniques. The objective of this study was to compare in vitro and in situ methods for determining rate and extent of forage digestion.

Materials and Methods

General

Three Angus-cross cattle (2 steers, 1 heifer; average BW = 1300 lb.) were fed chopped (2.54 cm) bromegrass hay (11.1% CP, 64.7% NDF) at 105% of maintenance (NRC, 1996) for 7 days to allow for diet adaptation. On day 8, duplicate polyester in situ bags (ANKOM Co., Fairport, NY; 10 x 20 mm with a 50- μ m pore size) containing 5.0 g of ground (2 mm) bromegrass hay for each incubation time were soaked in lukewarm water for 1 minute and inserted into the ventral rumen prior to feeding. In situ bags were serially removed at 3, 6, 9, 12, 15, 18, 24, 36, 48, and 96 hours after ruminal insertion and immediately frozen. Samples were later thawed at room temperature, rinsed in tepid water until rinse water remained clear, and dried in a 55°C forced-air oven. In vitro filter bags (ANKOM F57 filter bags; 25 μ m pore size) containing 0.5 g of bromegrass hay were incubated in an ANKOM Daisy^{II} incubator (ANKOM Technology, Fairport, NY) for incubation times as described for the in situ experiment above according to the methods described by the procedure of Tilley and Terry (1963). The ruminal fluid needed to inoculate the ANKOM incubator jars was collected after the last in situ bag was removed from the rumen in an effort to minimize potential ruminal disturbances associated with ruminal fluid collection.

In situ and in vitro residues were rinsed with NDF solution using an ANKOM 200 fiber analyzer (ANKOM Technology, Fairport, NY) to correct for possible microbial contamination (Van Soest et al., 1991). Residues were then analyzed for OM (AOAC, 1990) and CP (LECO model FP-528 Nitrogen Determinator, LECO, St. Joseph, MI) content to obtain an insoluble CP value.

Calculations and Statistical Analyses

Using the NLIN procedures of SAS (Release 7.0, ver. 4.1, 1998; SAS Institute, Cary, NC), the nonlinear model of Orskov and McDonald (1970) was used to calculate protein fractions A, B, and C, and protein degradation rate (k_d). Effective ruminal degradation (ERD) of forage protein was calculated using the equations of Broderick (1994), wherein $ERD = A + (B \times (k_d / (k_d + k_p)))$. Because the forage would most likely be associated with the particulate phase (Coblentz et al., 1999), the particulate passage rate estimate of Scholljegerdes et al. (2000; 4.9%/hr) in which the same cattle were fed similar forage was used for calculation of forage ERD. All resulting data were then subjected to ANOVA using the GLM procedures of SAS, and least squares means and SEM are shown in Figures 1 and 2 and Table 1.

Results and Discussion

Extent of OM digestion determined in situ was higher ($P \leq 0.03$) than in vitro at 15, 24, 36, and 96 hours (Figure 1A). Similarly, extent of NDF digestion was comparable ($P = 0.10$ to 0.44) between techniques at incubation times of 6 through 24 h, but differed ($P \leq 0.03$) at 3, 36, 48, and 96 hours of incubation (Figure 1B). Varel and Kreikemeier (1995) also observed that in situ estimates for NDF digestibility were consistently higher than those determined in vitro. A possible explanation of the differences in OM and NDF digestibilities could be due to different pore size between in situ and in vitro (50 μm and 25 μm , respectively) incubation bags. At the early time points, the smaller pore size may limit the infiltration of the ruminal bacteria into the in vitro bags; however, it is suggested that pore size be greater than 10 μm to prevent problems with microbial infiltration (Vanzant et al., 1998). Conversely, pore size for the in situ bags (50 μm) was twice that of the in vitro (25 μm) bags, which could potentially allow for greater particulate wash out when using the in situ technique (Nocek, 1985). The greater extent of digestion for the in situ technique at 36, 48, and 96 h may also be attributed to the sustained supply of nutrients to the microflora in the in situ technique as the animal continues to consume feed (animals were fed every 12 h). Conversely, no new dietary substrate is presented to the bacteria in the in vitro system, which could potentially limit the rate and extent of digestion due to limitations in specific nutrients, particularly at longer incubation times (Van Soest, 1994).

Estimates of N fractions A, B, and C did not differ ($P \geq 0.12$) for either technique (Table 1), which is attributable to the same forage being used in both techniques. This agrees with Uden and Van Soest (1984) who indicated that there were no differences between the A and B cell wall fractions when they compared in situ and in vitro techniques in heifers fed guinea grass. However, estimated CP degradation rate (k_d) in our experiment was eight fold higher ($P = 0.01$) for in situ (8.1%/hr) than in vitro (1.6%/hr) technique, which contributed to the greater ($P \leq 0.01$) extent

of forage CP digestion determined in situ than in vitro at all incubation times (Figure 2). Broderick et al. (1988) also observed higher estimates for k_d for in situ versus in vitro techniques. As with OM and NDF digestibilities, these results are likely related to the pore size difference between the two techniques. Nocek (1985) reported that CP disappearance from soybean meal increased linearly as bag pore size increased.

In this trial, the greater k_d associated with the in situ technique resulted in greater ($P = 0.0001$) forage protein ERD, or ruminally degradable protein (RDP) for the in situ (73.6%) compared to the in vitro (53.8%) technique (Table 1). When forage similar to that used in the current study was fed to beef heifers at 105% of maintenance, Scholljegerdes et al. (2001) reported a true ruminal CP digestibility of 53.4%, which is strikingly similar to the in vitro RDP estimate determined in the current study. Additionally, the marked difference between the ERD determined using the in situ technique and the true ruminal CP digestibility determined in vivo suggests that the in situ technique may overestimate forage RDP value. Broderick et al. (1988) also suggested that CP degradation estimates determined in vitro seem to be more closely related to in vivo estimates than those determined in situ. However, given the marginal differences between the two techniques in extent of OM and NDF digestion we concur with the suggestion of Varel and Kreikemeier (1995) that choice of technique should be based on that which is most convenient and should be governed by the research goal.

Implications

Digestibility estimates obtained from in situ and in vitro techniques can be highly variable depending on the nutrient examined. In particular, ruminal protein degradation estimates determined in vitro were more closely related to in vivo estimates than those determined in situ. Moreover, the marked difference in estimated forage protein degradability determined in situ from that determined in vivo suggests that the in situ technique may overestimate ruminal forage protein degradation. Although the disparity between techniques may be attributable to differences in pore size, more research is needed to examine the possible sources of variation between these techniques.

Literature Cited

- AOAC. 1990. Official Methods of Analysis (15th Ed.). Association of Official Analytical Chemists, Arlington, VA.
- Broderick, G. A. 1994. Quantifying forage protein quality. In: G. C. Fahey, Jr. (Ed.) Forage Quality, Evaluation, and Utilization. pp. 200-228. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, WI.

- Broderick, G. A., R. J. Wallace, E. R. Orskov, and L. Hansen. 1988. Comparison of estimates of ruminal protein degradation by in vitro and insitu methods. *J. Anim. Sci.* 66:1739-1745.
- Coblentz, W. K., J. O. Fritz, W. H. Frick, R. C. Cochran, J. E. Shirley and J. E. Turner. 1999. In situ disappearance of neutral detergent insoluble nitrogen from alfalfa and eastern gamagrass at three maturities. *J. Anim. Sci.* 77:2803-2809.
- Orskov, E. R., and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.* 92:499-503.
- Nocek, J. E., 1985. Evaluation of specific variables affecting in situ estimates of ruminal dry matter and protein digestion. *J. Anim. Sci.* 60:1347-1358.
- NRC. 1996. *Nutrient Requirements of Beef Cattle (7th Ed.)*. National Academy Press, Washington, DC.
- Scholljegerdes, E. J., B. W. Hess, and P. A. Ludden. 2000. The influence of level of feed restriction on forage digestibility by beef cattle. *Proc. West. Sect. Amer. Soc. Anim. Sci.* 51:86-90.
- Scholljegerdes, E. J., J. Gould, B. W. Hess, and P. A. Ludden. 2001. Site and extent of digestion in beef cattle consuming restricted amounts of forage and supplemental undegradable intake protein. *Proc. West. Sect. Amer. Soc. Anim. Sci.* 52:463-468.
- Tilley, J. M. A. and R. A. Terry. 1963. A two stage technique for the in vitro digestion of forage crops. *J. Brit. Grassl. Soc.* 18:104-111.
- Uden, P. and P. J. Van Soest. 1984. Investigation of the in situ bag technique and a comparison of the fermentation in heifers, sheep, ponies and rabbits. *J. Anim. Sci.* 58:213-221.
- Van Soest, P. J. 1994. *Nutritional Ecology of the Ruminant.* Cornell Univ. Press. Ithaca, NY.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597.
- Vanzant, E. S., R. C. Cochran, and E. C. Titgemeyer. 1998. Standardization of in situ techniques for ruminant feedstuff evaluation. *J. Anim. Sci.* 76:2717-2729.
- Varel, H. V., and K. K. Kreikemeier. 1995. Technical Note: Comparison of in vitro and in situ digestibility methods. *J. Anim. Sci.* 1995. 73:578-582.

Table 1. A comparison of techniques to estimate forage N fractions, k_d , and ruminal degradability.

		In situ	In vitro	SEM ^a	P<
CP Fractions, % of total CP					
	A	46.8	41.63	2.54	0.2223
B	43.6	53.62	3.63	0.1225	
C	9.60	4.75	3.41	0.3712	
k_d , %/hr	8.09	1.55	0.97	0.0087	
RDP ^b	73.63	53.84	0.84	0.0001	

^an = 3

^bRDP = Ruminally Degradable Protein.

Figure 1. Comparison of in vitro (■) versus in situ (●) techniques used to determine extent of A) OM and B) NDF digestion at incubation times up to 96 h. Means (\pm SEM) within incubation time with superscripts differ (* $P \leq 0.05$; † $P \leq 0.01$).

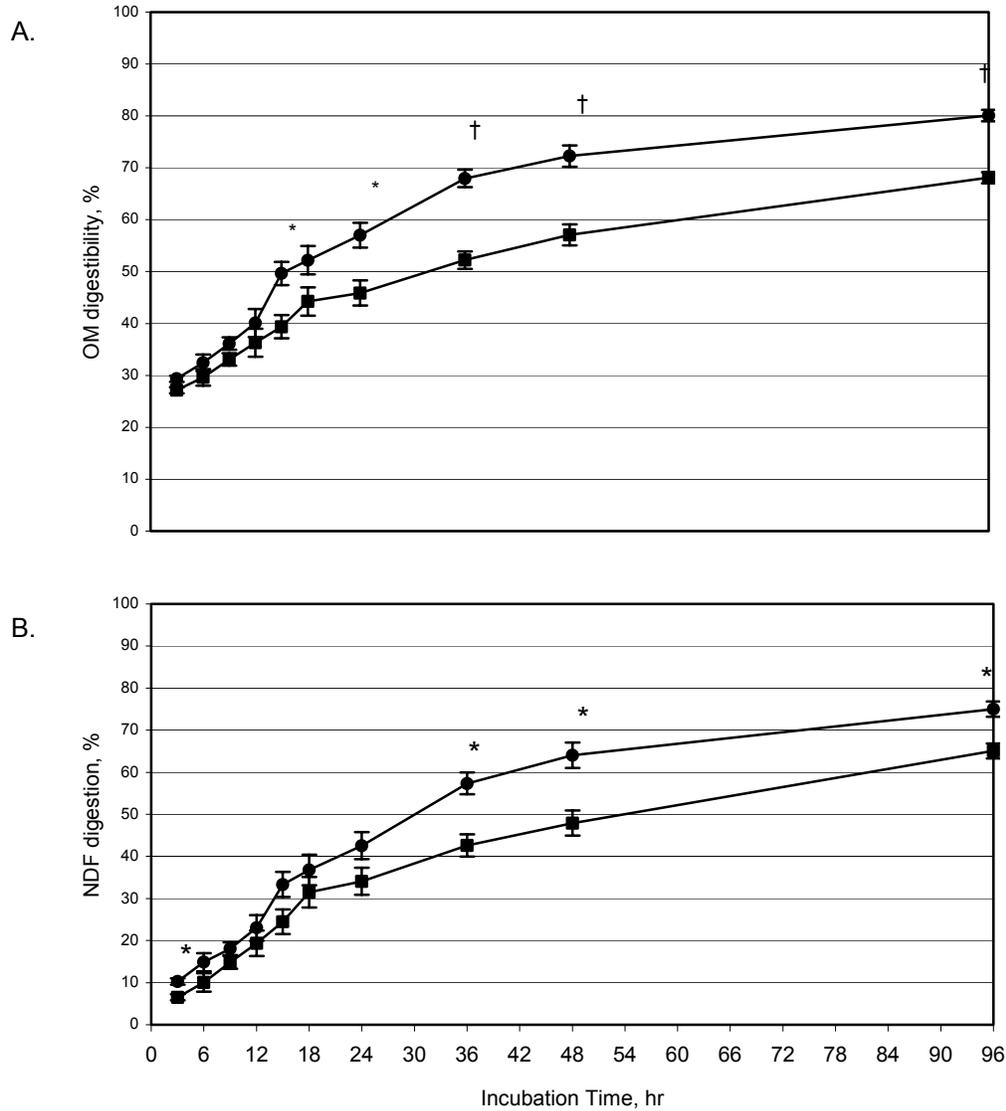


Figure 2. Comparison of in vitro (■) versus in situ (●) techniques used to determine extent of CP digestion at incubation times up to 96 h. Means (\pm SEM) within incubation time with superscripts differ (* $P \leq 0.05$; † $P \leq 0.01$; ‡ $P \leq 0.001$).

