Use of Pepsin-Cellulase for Estimating Forage Nutritive Value

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Summary

An experiment was conducted in 1986 to determine the feasibility of replacing rumen fluid with fungal cellulase. Comparisons were made between in-vivo, in-vitro, and cellulase digestibility. Highly significant correlations were observed between in-vivo digestibility (IVD) and in-vitro (IVDMD) (r = .95**), as well as between IVD and cellulase solubility (CDMD) (r = .97**). Correlations were also highly significant between IVDMD and CDMD (r = .94**). Results suggest that cellulase solubility may replace traditional in-vitro analysis for warm-season perennial grasses.

Introduction

In-vitro analysis (IVDMD) to predict forage in-vivo (IVD) digestibility has been widely utilized by plant breeders and agronomists to estimate forage nutritive value. While the technique has been widely applied, numerous problems are associated with it.

Of primary concern to the agronomist is that a donor animal (fistulated steer) must be maintained in order to provide a source of rumen fluid. Numerous studies have addressed the variability associated with rumen fluid and results obtained with IVDMD analysis (Clark and Mott, 1960; Van Dyne, 1962; Drew, 1966; Troelsen and Hansel, 1966; Minson and McLoed, 1972). Due to this variation, alternate methods of estimating forage IVD are required.

The use of cellulase enzymes as a replacement for rumen fluid was first proposed in the mid-1960's, however, the initial cellulase preparations were too pure to provide good relationships with in-vivo samples. In recent years, numerous studies have been reported in which good relationships have been obtained between fungal cellulase digestion and in-vitro or in-vivo digestibilities (Bughara and Sleper, 1986; Jones and Hayward, 1975).

The purpose of this study was to evaluate a commer-

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cially available source of cellulase for its effectiveness in estimating forage IVDMD and IVD.

Procedure

An experiment was conducted to evaluate the relationship between IVD or IVDMD and pepsin-cellulase solubility (CDMD). For all experiments IVDMD was determined according to the methods of Goering and Van Soest (1970) and pepsin-cellulase solubility was determined by a modification of the methods of Jones and Hayward (1973). For this study, 0.2 grams of forage were incubated in 30 ml of acid-pepsin for 24 hours, the supernatant removed by suction through gas dispersion tubes (coarse porosity), and 30 ml of 1 percent (v/v) buffered cellulase added.

Treatment 1 (CDMD-1) consisted of a 24-hour cellulase incubation while treatment 2 (CDMD-2) consisted of a 5-hour incubation, removal of the cellulase solution, and 19 hours in fresh cellulase solution. The samples utilized in this study (Table 1), consisted of 14 samples of known in-vivo digestibility (47.4 to 72.4 percent). In-vitro analysis and CDMD were compared by regression analysis and using Spearmann Rank Correlations.

Results and Discussion

Initial comparisons between IVD and CDMD yielded highly significant r^2 values (Table 2), while Spearmann Rank correlations indicated that CDMD ranked forage samples as well as in-vitro analysis (Table 3). However, a close look at the spread of the IVD samples revealed that two points were clustered at about 72 percent IVD while the remainder of the samples (12) had values between 47 and 60 percent (Table 3). For this reason, the two points were removed and the data set reanalyzed. The removal

TABLE 1. IN-VIVO SAMPLES UTILIZED IN PEPSIN-CELLULASE STUDY

Sorghum-sudans	Bermudagrasses	Ryegrasses
47.35	48.31	72.00
48.62	49.14	72.37
50.50	51.21	
53.64	57.45	
53.87	60.18	
55.04		
56.03		

TABLE 2. RELATIONSHIP BETWEEN CDMD-1, CDMD-2, IVDMD, AND *IN VIVO* DIGESTIBILITY

		r^2
CDMD-1	$Y = 31.62 + .56x$	$r^2 = .94^{**}$
CDMD-2	$Y = 31.40 + .53x$	$r^2 = .94^{**}$
IVDMD	$Y = 15.87 + .64x$	$r^2 = .91^{**}$

TABLE 3. SPEARMANN RANK CORRELATIONS FOR CDMD-1, CDMD-2, IVDMD, *IN-VIVO* DIGESTIBILITY

	n = 14	n = 12
CDMD-1	.89**	.84**
CDMD-2	.88**	.80**
IVDMD	.85**	.76**

of the ryegrass samples reduced the magnitude of the correlation coefficients as well as the coefficients of determination (r^2) (Table 4). Even though the magnitude of the correlation coefficients was reduced, the relationship between CDMD and IVD was better than the relationship between IVDMD and IVD. This suggests that cellulase may replace rumen fluid to determine the relative digestibility of warm-season grasses.

Caution should be used in extrapolation from this data set since only a single source of cellulase was utilized. It has been reported that cellulase sources differ widely in their activities and sources with a high endo- : ectocellulytic activity ratio are generally preferred. Results from Bughara and Sleper (1986) suggest that B values as high as 1.00 may be obtained when higher activity cellulases are utilized, and thorough comparison of cellulase sources should be made prior to recommending the use of a single source.

Based on experience in our lab, errors associated with CDMD are less than those for in-vitro analysis. To date, we have analyzed approximately 6,000 samples using pepsin-cellulase and have yet to have the bermudagrass standards deviate by more than two digestibility units between runs.

TABLE 4. PEARSON CORRELATION COEFFICIENTS AND R^2 VALUES FOR CDMD-1, CDMD-2, IVDMD, AND *IN-VIRO* DIGESTIBILITY

	Pearson coefficients		r^2
	n = 14	n = 12	n = 12
CDMD-1	.97**	.85**	.73**
CDMD-2	.97**	.86**	.73**
IVDMD	.95**	.82**	.66**

**—Denotes correlation significant at the .001 level for all tables.

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