

A Comparison of Microbial Cellulase and Live Cell Rumen Inoculum for Estimating *In Vitro* Digestibility of Range Grasses

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ABSTRACT

Three commercial-grade cellulase enzymes and a live cell rumen inoculum were used to compare *in vitro* digestible dry matter of five high cellulose grass species in advanced stages of maturity in the Trans-Pecos, Texas. Three prediction equations were developed to define the relationships between *in vitro* rumen inoculum techniques and those using cellulase enzymes. All microbial cellulase techniques reliably estimated digestibility, although the rumen inoculum provided a greater extent of digestibility ($P \leq 0.01$) than the cellulases. Different grass species exhibited varied levels of digestibility ($P \leq 0.01$). Prediction equations between the rumen fluid and cellulase techniques were well correlated ($r^2 = 0.69$ to 0.81) and highly significant ($P \leq 0.01$). These data suggest that enzyme digestibility estimates are effective and may be accurate predictors of live cell dry matter digestibility of range grasses consumed by ruminants.

KEY WORDS: alkali sacaton, Arizona cottontop, blue grama, IVDDM, Johnsongrass, sideoats grama, Trans-Pecos

Digestibility of feedstuffs containing cellulose is an important nutritive aspect of range forages. It is useful for livestock producers to know nutritive values of specific forages to apply effective grazing management and to improve supplemental feeding management for grazing animals. The method developed by Tilley and Terry (1963) is a standard procedure to assess *in vitro* digestible dry matter (IVDDM) of forages. This method, however, requires a readily available source of fresh rumen fluid from a ruminant fed a forage consistent with the samples to be analyzed. Rumen fistulated animals are expensive to obtain and maintain (Goto & Minson, 1977), and rumen fluid activity varies when taken at different times and from animals grazed on different forages. This variation results in poor precision (Tilley & Terry, 1963).

Recently developed methods involving microbial cellulase could be used to replace rumen fluid as the digestion media for *in vitro* digestibility assessments (Bughrara et al., 1985; Gabrielsen, 1986; Dickerson et al., 1988). The use of commercially

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available microbial cellulase as a digestion media would eliminate the need for ruminally fistulated animals, and potentially provide a reliable and repeatable method to analyze grazeable forages (Donefer et al., 1963). Microbial cellulases give accurate predictions of dry matter digestibility (Bughrara et al., 1985; Gabrielsen, 1986), but may not be as precise when used on coarse, low-quality forage (Bughrara et al., 1985; Dickerson et al., 1988). Marten et al. (1988) reported that immature plants are subject to more complete digestion with cellulase enzymes than mature forages.

The objectives of this study were to: 1) test different cellulase sources for repeatable cellulolytic activity for determination of IVDDM of forage grasses; 2) assess differences in digestibility estimates among selected range grasses; and 3) correlate IVDDM estimates of microbial cellulase solutions with those made with live cell rumen fluid inoculum to assess the utility of microbial cellulase digestion media as a predictor of dry matter digestibility.

MATERIALS AND METHODS

Five grasses were selected for analysis based on their regional abundance and importance as forage species in the Trans-Pecos of Texas: sideoats grama [*Bouteloua curtipendula* (Michx.) Torr.]; blue grama, [*Bouteloua gracilis* (H.B.K.) Lag. ex Steud.]; Arizona cottontop [*Digitaria californica* (Benth.) Henr.]; Johnsongrass [*Sorghum halepense* (L.) Pers.]; and alkali sacaton [*Sporobolus airoides* (Torr.) Torr.]. Six individual plants of each species were collected at maturity on 3 October 1988 northeast of Alpine, Texas, and on 2 November 1988 on the Nation's Ranch between Fort Davis and Balmorhea, Texas. Each plant was clipped at ground level, placed in a paper bag and air dried. Plants were ground in a Wiley mill to pass a 0.040 in. mesh screen, and composite samples were made for each species and thoroughly mixed.

Percent IVDDM of the composite samples was determined using three different cellulase enzymes and rumen fluid. Commercially available cellulases derived from *Penicillium funiculosum* and *Aspergillus niger* were used, as well as Cabisco Chemicals No. 85-3630. Rumen fluid was obtained from a ruminally fistulated steer grazed on mature standing forage in pastures with botanical composition comparable to those pastures sampled for grasses.

For the cellulase enzyme treatment the procedure outlined by Dowman and Collins (1982) was followed. This procedure was modified by using a consistent level (0.70 oz./qt.) of each cellulase, with 0.007 oz. of each forage sample, instead of varying the amounts of cellulase enzyme. A constant level of cellulase enzyme may affect the efficacy of one enzyme more than others, but provides a baseline to compare each treatment. The *in vitro* digestibility technique with rumen fluid inoculum followed procedures outlined by Ellis (unpublished), using 0.018 oz. samples of each forage. For each technique, duplicate subsamples, together with duplicates of a standard forage (oat hay) were analyzed. Each subsample pair was averaged and treated as a single observation. Only pairs with less than $\pm 3\%$ difference from the pair mean were used as an observation, and four replicate observations were analyzed for each combination of grass species and cellulase enzyme. Digestibilities were corrected for bias using the standard forage as outlined by McLeod and Minson (1979).

Analysis of variance and Duncan's new multiple range test were used to identify

significant differences ($P \leq 0.01$) among species and digestion treatments. Linear regression and correlation procedures were performed to determine predictability of cellulase digestibilities relative to rumen fluid inoculum. Samples of all species were combined for each source of cellulase enzyme providing a sample size of 16 for each regression analysis. All analyses were performed using MSTAT (Freed et al., 1987).

RESULTS AND DISCUSSION

Each digestion media had an apparent cellulolytic effect on the mature forages tested, and each dry matter digestibility was significantly different ($P \leq 0.01$) when compared using one-way analysis of variance. Rumen fluid inoculum was most effective (49%), followed by *P. funiculosum* (43%), Cabisco No. 85-3630 (39%) and *A. niger* (33%). *Aspergillus niger* was the least effective enzyme source, in contrast with results from Clark and Beard (1977) who reported that *A. niger* was able to degrade a wide variety of substrates. Extent of digestion was greater for rumen fluid inoculum than for the cellulase enzymes, but all sources exhibited similar efficiencies of digestibility among the grass substrates (Table 1).

Table 1. Mean percent digestibilities (\pm SE) for each grass species within each digestion media.

Enzyme Source	Johnsongrass	Arizona cottontop	Sideoats grama	Blue grama	Alkali sacaton
	----- % -----				
Rumen Fluid	69.27 (0.57)	53.20 (0.27)	47.48 (0.62)	38.12 (1.41)	37.23 (0.86)
<i>Penicillium funiculosum</i>	55.74 (1.58)	42.78 (2.29)	40.73 (0.91)	39.63 (1.32)	34.35 (1.98)
Cabisco Chem. No. 85-3630	52.53 (0.86)	38.12 (1.62)	37.51 (0.29)	36.53 (1.41)	25.08 (1.87)
<i>Aspergillus niger</i>	45.93 (1.89)	30.97 (0.20)	31.87 (0.54)	32.26 (3.40)	30.66 (1.02)

Johnsongrass (55%) was significantly more digestible than other grasses. Arizona cottontop (41%) and sideoats grama (40%) were intermediate in IVDDM whereas blue grama (37%) and alkali sacaton (32%) were significantly less digestible than the other species. A two-way analysis of variance of the data produced a digestion media by grass species interaction, demonstrating a forage-specific response to different cellulase sources. This indicated that certain cellulases may be more effective in hydrolyzing dry matter in particular grasses and suggests that within a comparison trial, the same digestion media and technique should be applied to all samples for results to be comparable.

Estimates of IVDDM using each cellulase source were significantly correlated ($P \leq 0.01$) with the IVDDM estimates using the rumen fluid source (Table 2). The coefficients of determination were lower than those found by Bughrara et al. (1985), but their values were derived for individual grass species, rather than combined species. In addition, grasses used in the current study were coarse, mature warm

season species, recognized as having low digestibilities (Barton et al., 1976; Dickerson et al., 1988). Results of this study were comparable to those found for mixed species composition analysis (Dickerson et al., 1988), which is more representative of rangeland grazing conditions.

Table 2. Regression equations and coefficients of determination (r^2) for three cellulase enzymes predicting digestibility based on live cell rumen inoculum IVDDM.

Cellulase enzyme	Regression	r^{2**}
<i>Penicillium funiculosum</i>	$Y = -9.99 + 1.384x$	0.81
Cabisco No. 85-3630	$Y = -6.06 + 1.410x$	0.80
<i>Aspergillus niger</i>	$Y = 6.37 + 1.285x$	0.69

**All coefficients of determination significant ($P < 0.01$).

Correlations ($r = 0.83$ to 0.91) among the three cellulase sources and rumen fluid were also significant ($P \leq 0.01$). Because cellulases hydrolyze the same beta-bonds in cellulose, correlations should be high; however, efficacy of different cellulases would be expected to vary. The regressions in Table 2 indicate that the cellulase enzymes used provided reliable predictors of rumen fluid digestibility for mature warm season range grasses. These results, as well as those of others (Donefer et al., 1963; McLeod & Minson, 1982; Bughrara et al., 1985; Dickerson et al., 1988), suggest that the use of cellulase enzymes can provide accurate and lower cost evaluations of IVDDM than the use of rumen fluid.

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