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Crop Quality and Utilization: A Twelve-Hour In Vitro Procedure for Sorghum Grain Feed Quality Assessment

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CROP QUALITY & UTILIZATION

A Twelve-Hour In Vitro Procedure for Sorghum Grain Feed Quality Assessment

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ABSTRACT

Improved methods for assessing cereal crop feed value are a prerequisite for the genetic improvement of sorghum [*Sorghum bicolor* (L.) Moench] feed value. Rate of starch digestion is now commonly believed to be the limiting factor in sorghum utilization by cattle (*Bos taurus*). However, techniques to assess this trait are not useful to sorghum breeders because of high labor inputs, lab error associated with starch measurement, and need for high numbers of replications. The objective of this study was to develop a simple technique capable of identifying differences in digestion between sorghum and corn (*Zea mays* L.) and detecting differences among sorghum genotypes. In vitro starch and dry matter digestion were measured on sorghum and corn lab standards at 0, 6, 12, 18, 24, 30, and 40 h. Maximum differentiation between corn and sorghum dry matter digestion (345 vs. 253 g kg^{-1}) and starch digestion (403 vs. 301 g kg^{-1}) occurred at 12 h, and dry matter and starch digestion were highly correlated (r = 0.99). Differences among five sorghum lines were significant for 12-h dry matter digestion and ranged from 229 to 272 g kg^{-1}. This procedure provides a precise and rapid technique that can be used by feed grain breeders to evaluate modifications in grain digestion parameters.

The major U.S. sorghum consumer, the cattle feedlot industry, recognizes one primary utilization problem with sorghum: lower feed value than corn. These consumers assess proportionally lower cash value to sorghum grain. The literature is somewhat ambiguous concerning the actual feed value of sorghum. Depending on physical processing of grain prior to feeding, sorghum has been shown to be 85 to 100% of the value of corn in livestock production (National Research Council, 1996). However, it is generally believed in the feedlot industry that sorghum grain is 5 to 10% lower in feed value (efficiency) than corn. Cash value of sorghum grain averages ≈90% that of corn (USDA Agricultural Statistics, 1994).

Improved methods for assessing cereal crop feed value are a prerequisite for the genetic improvement of sorghum feed value. During the past 30 yr, researchers have described numerous factors known, or believed, to impact feed quality. These include endosperm color (Noland et al., 1977; Streeter et al., 1991), endosperm type (Lichtenwalner et al., 1978; Sherrod et al., 1969), endosperm texture (Cohen and Tanksley, 1973; Elmalik et al., 1986; Samford et al., 1971), pericarp color (McCollough et al., 1972; Noland et al., 1977), protein composition (Singh and Axtell, 1973; Deyoe and Shellenberger, 1965), and protein digestibility (Axtell et al., 1981; Bramel-Cox et al., 1995).

Several techniques for assessing sorghum grain feed value specifically for ruminants (cattle) have also been described utilizing actual rumen fluid to digest samples. Due to the complexities of the ruminant digestive system, such approaches are intuitively better predictors of feed value. Miller et al. (1972) tested nylon bag and in vitro dry matter digestion (IVDMD) systems and identified genetic differences in grain quality of sorghum, but concluded that "none of the methods studied appear good enough to predict animal performance." Hibberd et al. (1982a) showed a wide range in IVDMD in nine sorghum lines with varying endosperm type and bird resistance. In a later study, Hibberd et al. (1982b) showed little difference in IVDMD of purified starch from the same nine lines.

A series of papers by Russell et al. (1992), Sniffen et al. (1992), and Fox et al. (1992) discuss the Cornell Net Carbohydrate and Protein System. This system estimates rumen fermentation and end-products on the basis of a number of factors including both chemical makeup of feedstuffs and class of livestock being fed. Although their system is somewhat complex for direct use by a plant breeding project, Sniffen et al. (1992) does identify a key difference between sorghum and corn: the rate of starch digestion (5–15 vs. 10–20% h^{-1}, respectively).

Wester et al. (1992) showed genetic variation in sorghum for in vitro rate of starch disappearance (IVRSD) and demonstrated a strong relationship between IVRSD and feed/gain ratio (R^2 = 0.94) in the feedlot. Rate of starch digestion is now commonly believed to be the limiting factor in sorghum utilization by cattle. Use of IVRSD in sorghum breeding has proven difficult due to high lab error rates associated with starch measurement. Considerable IVRSD technique refinement has been accomplished (Richards et al., 1995), but the technique has not been useful to sorghum breeders because of high labor inputs, lab error associated with starch measurement, and the need for high numbers of replications.

Recent efforts in our lab have focused on using a sealed filter bag/bulk IVDMD fermenter (ANKOM

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Abbreviations: IVDMD, in vitro dry matter digestion; IVRSD, in vitro rate of starch disappearance.

Technology Corp., Fairport, NY). For forage analysis, the ANKOM fermenter was shown to accurately discriminate among genotypes, have low lab error, and greatly simplify sample handling and processing (Vogel et al., 1999).[1]

The objectives of this study were to develop an IVDMD procedure to measure differences between sorghum and corn digestion using the ANKOM fermenter, and to utilize that procedure to differentiate among sorghum genotypes.

MATERIALS AND METHODS

During the development of the techniques used in this research, results were often inconclusive or at times contradictory. Examination of possible procedural sources of error caused us to conclude that sample preparation and sampling were of critical importance in obtaining repeatable results. Samples analyzed for this report were ground within 1 wk of laboratory analysis and special care was taken to prevent stratification of ground samples prior to subsampling.

Experiment 1—Digestion of Grain Components at 6, 12, 18, 24, 30, and 40 Hours

The sorghum standard (white seed, bin run mixture of 'Goldenharvest 388', 'Agripro 3502', and 'NC+ 7C49') and corn standard ('Pioneer 3394') were ground to pass a 2-mm screen in a Wiley mill. Ninety-six 0.5-g subsamples of each were sealed in preweighed ANKOM F57 filter bags (95% of pores <30 microns, other 5% <50 microns), dried at 60°C for 24 h, and weighed to determine sample dry weight. Twelve subsamples of each species and a sealed blank filter bag were then placed in each of eight 3.875-L glass vessels.

Rumen fluid inoculum was obtained whole from a ruminally fistulated steer maintained on a high concentrate diet containing 79% dry rolled corn, 10% alfalfa hay, 4% Liquid 32 (Liquid Feed Commodities, Fremont, NE), 2% molasses, and 5% dry supplement. Except where modified for the bulk glass digestion vessels, inoculum collection and filtering procedures and solutions were as described for the direct acidification method with Kansas State buffer (Marten and Barnes, 1980). Buffer solution (1600 mL) and rumen inoculum fluid (400 mL) were added to each vessel, the vessels were purged with CO_2, and lids with gas relief valves were placed on the vessels.

The glass fermentation vessels were then placed into two ANKOM Rumen Fermenters Model No: Daisy II (four vessels each) and incubated while continuously rotating at 39° C. At 6 h, one vessel was removed. Filter bags were rinsed four times with warm tap water, dried at 60° C for 48 h, and weighed. Subsamples were then bulked by species to yield sufficient quantities of digested samples for subsequent analyses. Vessels also were removed at 12, 18, 24, 30, and 40 h and treated similarly, except that the contents of two vessels were bulked by species for the 30- and 40-h digestion periods.

Starch contents of the undigested sorghum and corn, and the sorghum and corn bulk residues after 6-, 12-, 24-, 30-, and 40-h digestion were determined as α-linked glucose polymers by a modified MacRae and Armstrong (1968) procedure as described by Richards et al. (1995). Crude protein content was determined by the combustion method (Association of Official Analytical Chemists, 1996) and corrected for microbial contamination using purines as a marker (Zinn and Owens, 1986).

The experiment was replicated three times in a randomized complete block design. Unlike experiments with the objective of describing the mechanics or kinetics of the rumen, the objective or this experiment was to identify a simple in vitro procedure to detect differences between sorghum and corn digestion using the ANKOM fermenters. Single degree of freedom comparisons between sorghum and corn were made for component digested at the end of each period. Simple correlations among dry matter, starch, and crude protein digested were also calculated.

Since our earlier research demonstrated that in vitro sorghum starch digestion begins to plateau at 12 h of digestion (Richards et al., 1995), and grain concentrates such as sorghum are thought to pass the rumen in ≈12 h (Sniffen et al., 1992), 12-h digestion rates were estimated from 6- and 12-h data using linear regression after forcing the intercept to zero (SAS Institute, 1990). Quadratic regression equations were also fit for 0 to 40 h and results plotted.

Experiment 2—Twelve-Hour In Vitro Dry Matter Digestion of Five Sorghum Lines

The five sorghum conversion lines used in this study were selected for known diversity in grain quality parameters (Table 1) and were also used by Richards et al. (1995) in studies on starch digestion. Entries were seeded at the University of Nebraska Field Laboratory in Ithaca, NE (Sharpsburg silty clay loam [fine montmorillonitic, mesic Typic Agriudoll]) in 7.6-m rows spaced 76 cm apart in a randomized complete block with four replications each year. Planting dates were 21 May 1991 and 13 May 1992. Plots were fertilized with 112 kg ha^{-1} N prior to planting. For weed control, propachlor [2-chloro-*N* (1-methylethyl)-*N*-phenylacetamide] and atrazine [6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5,-triazine-2.4.-diamine] were applied at 3.36 and 1.12 kg ha^{-1}, respectively, immediately after planting. No supplemental irrigation was applied. Plots were harvested with a small-plot combine and grain was stored at 8°C until used in this study.

Grain preparation and in vitro digestion procedures were as above with the following modifications. Digestion was for a 12-h period only, and dry matter digested was the only parameter reported. The experimental design was a randomized complete block with four replications in each of the 2 yr. All samples were run in quadruplicate, with these being treated as repeated observations in the analyses. Analysis of variance and the F-protected Duncan's multiple range test were used to test for differences among lines.

Table 1. Previously established seed parameters of five sorghum conversion lines. Experiment 1†.

Line	SKWCS‡ hardness	Vitreous endosperm	Diameter	Weight	Density	IVRSD§
		%	mm	mg	g cm^{-3}	% h^{-1}
SC203	78c¶	62c	4.33a	42.9a	1.392b	7.9a
SC215	92b	68bc	3.45b	24.8c	1.390b	7.2a
SC242	108a	86a	2.71d	16.4d	1.418a	6.4a
SC460	67d	65c	4.17a	30.1b	1.373c	8.2a
SC835	97b	72b	3.19c	18.5d	1.399c	6.0a

† Adapted from Richards et al. (1995) and Pedersen et al. (1996).
‡ SKWCS = Single Kernel Wheat Characterization System. Hardness is expressed on a relative scale based on soft wheat = 50, hard wheat = 100.
§ IVRSD = In vitro rate of starch digestion using rumen fluid from a steer maintained on a high concentrate (corn) diet similar to that used in this study.
¶ Values within a column followed by the same letter are not different at $P < 0.05$ using Duncan's new multiple range test.

[1] Mention of a trademark, proprietary product, or a vendor does not constitute a guarantee or warranty of the USDA or the University of Nebraska and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Table 2. Dry matter, starch, and crude protein digested following 0, 6, 12, 24, 30, and 40 h of ANKOM in vitro dry matter digestion. Exp. 1.

Hour	Species	Proportion component digested					
		Dry matter + SE		Starch + SE		Crude protein + SE	
		g kg^{-1}					
6	Sorghum	126**	15	173	90	70	38
	Corn	188	14	201	80	106	112
12	Sorghum	253**	34	301**	31	60	60
	Corn	345	29	403	25	170	93
18	Sorghum	368*	12	437	24	99	36
	Corn	452	29	486	35	257	103
24	Sorghum	528	82	598	65	280	90
	Corn	591	41	627	34	435	125
30	Sorghum	622	37	693	26	342	70
	Corn	664	24	720	16	517	50
40	Sorghum	721	26	791	64	457	10
	Corn	742	21	801	72	499	67

*, ** Significant between sorghum and corn within a time period at the 0.05 and 0.01 probability levels, respectively.

Experiment 3—Forty-Eight-Hour In Vitro Dry Matter Digestion of Five Sorghum Lines

Grain from Exp. 2 was prepared and analyzed as in Exp. 2 except that digestion was for a 48-h period only. The value reported was dry matter digested at 48 h.

RESULTS

Experiment 1—Digestion of Grain Components at 6, 12, 18, 24, 30, and 40 Hours

Differences ($P \leq 0.05$) between the corn and sorghum standards were detected only for dry matter digested following 6, 12, and 18 h, and for starch digested after 12 h (Table 2). The ability to preferentially detect differences in dry matter digestion during the early fermentation periods is probably due to the low error associated with dry matter measurements, as well as maximal differences between sorghum and corn digested during these periods. Likewise, detection of a difference between sorghum and corn starch digestion after 12 h reflects maximum differences between sorghum and corn starch digestion, and low associated error. Since grain concentrate is thought to pass the rumen in ≈12 h (Sniffen et al., 1992) and since differences between sorghum and corn were large and detectable for both dry matter and starch digested after 12 h, we concluded that 12 h of digestion provides an opportunity to differentiate and assess the value of feed grains. The 12-h digestion rates were shown to differ ($P \leq 0.05$) between corn and sorghum dry matter (2.90 ± 0.11% h^{-1} vs. 2.07 ± 0.11% h^{-1}, respectively), and corn and sorghum starch digested (3.58 ± 0.23% h^{-1} vs. 2.80 ± 0.23% h^{-1}, respectively).

Table 3. Correlation of dry matter, starch, and protein digested using data from all digestion periods (†) and using a subset of data from the 6- and 12-h digestion periods (‡).

	Dry matter	Starch	Crude protein
Dry matter		0.99**†	0.91**†
Starch	0.81**‡		0.89**†
Protein	NS‡	NS‡	

** Significant at the 0.01 probability level; NS is not significant at the 0.05 probability level.

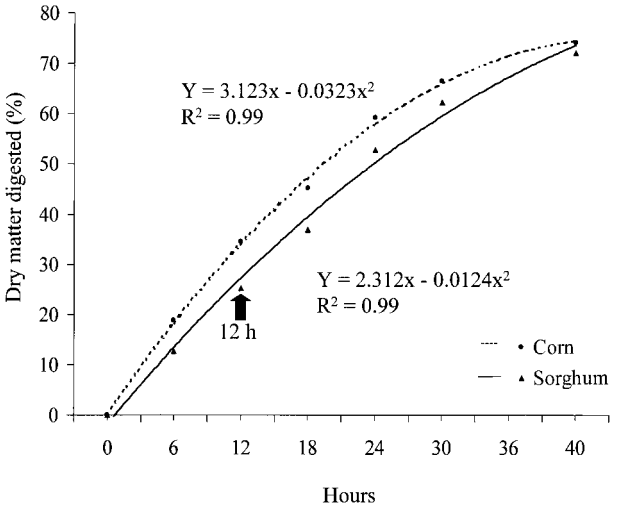

Fig. 1. Prediction of ANKOM in vitro dry matter digestion for sorghum and corn. Exp. 1.

Of the parameters considered, measurement of dry matter is the simplest and the most precise. Whether considering the entire data set (data from all digestion periods), or the data subset for the first 12 h (data from the 6- and 12-h digestion periods), the relationship between dry matter and starch digested is sufficiently strong ($r = 0.99$ and 0.81, respectively) to suggest that measurement of dry matter digestion will predict starch digestion (Table 3). During the first 12 h, protein digested was not correlated ($P > 0.05$) with either starch or dry matter digested.

To better understand and visualize the relationships of time and differential digestion of sorghum and corn in the ANKOM in vitro system, regression equations were fit for the entire 0- to 40-h period and plotted for dry matter and starch (Fig. 1 and 2). Corn dry matter and starch digested more rapidly than sorghum dry matter and starch during the first 12 h. During the 12- to 30-h period, sorghum and corn appeared to digest at

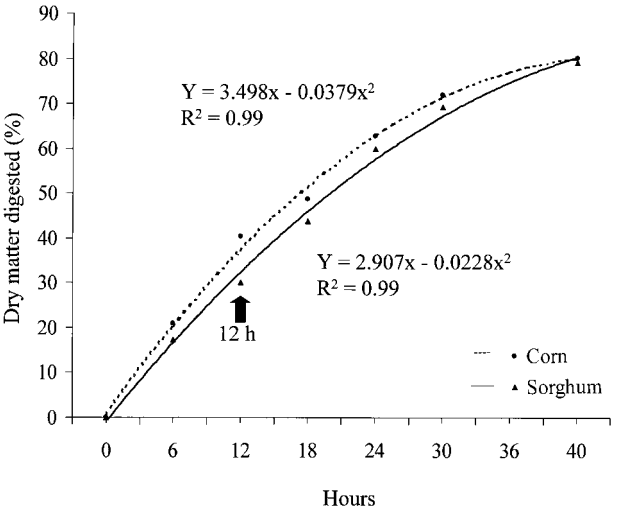

Fig. 2. Prediction of ANKOM in vitro starch digestion for sorghum and corn. Exp. 1.

Table 4. ANKOM 12-h in vitro dry matter digestion (IVDMD) for five sorghum lines. Exp. 2.

Sorghum line	IVDMD	± SE
	g kg^{-1} DM	
SC460	272a†	32
SC242	246b	52
SC215	236b	43
SC835	233b	37
SC203	229b	51
Sorghum standard‡	282	57
Corn standard	356	61

† Values within a column followed by the same letter are not different at $P < 0.05$ using Duncan's new multiple range test.
‡ Lab standards include as a reference for the reader's convenience and not for designed experimental comparisons.

Table 5. ANKOM 48-h in vitro dry matter digestion (IVDMD) for five sorghum lines. Exp. 3.

Sorghum line	IVDMD	± SE
	g kg^{-1} DM	
SC460	871a†	24
SC242	863a	26
SC215	863a	23
SC835	866a	21
SC203	868a	28
Sorghum standard‡	886	24
Corn standard	916	37

† Values within a column followed by the same letter are not different at $P < 0.05$ using Duncan's new multiple range test.
‡ Lab standards include as a reference for the reader's convenience and not for designed experimental comparisons.

approximately the same rates. Given adequate digestion time (40 h), extent of sorghum and corn dry matter and starch digestion become very similar. These graphical summaries reinforce the conclusion that differences in digestion between these sorghum and corn samples occur during the first 12 h of digestion.

Experiment 2—Twelve-Hour In Vitro Dry Matter Digestion of Five Sorghum Lines

In a second experiment, the sorghum conversion lines SC203, SC215, SC242, SC460, and SC835 grown in each of 2 yr were digested for a single 12-h period. Bartlett's test of homogeneity of variances (Steel and Torrie, 1960) indicated no differences, and data were pooled among years. Significant differences in 12-h IVDMD were detected among the lines (Table 4) indicating the presence of genetic variation for this trait. Within this small set of lines, 12-h IVDMD values ranged from 229 to 272 g kg^{-1}. These same five lines (using the same seed lots) were previously evaluated for extent or rate of starch disappearance in several experiments following 8 h of digestion using a modified Tilley and Terry (1963) procedure (Richards et al., 1995). Ranking of lines for starch disappearance was similar to our ranking for 12-h IVDMD. However, differences among lines for starch disappearance were not significant at $P \leq 0.05$ in any of the experiments reported by Richards et al. (1995).

Experiment 3—Forty-Eight-Hour In Vitro Dry Matter Digestion of Five Sorghum Lines

The above five sorghum lines were digested for 48 h in a separate experiment. As above, a Bartlett's test was performed, and data pooled among years. No differences were detected among lines (Table 5). These results represent the maximum extent of digestion in these experiments. Given adequate digestion time, all samples were digested similarly and thoroughly (IVDMD = 866 g kg^{-1}, SE = 25 g kg^{-1}) This again reinforces the earlier conclusions that critical differences in digestion are detectable and occur in the early (first 12 h) digestion time periods.

DISCUSSION

Current literature such as that describing the Cornell Net Carbohydrate and Protein System (Russell et al., 1992; Sniffen et al., 1992; and Fox et al., 1992) and the collective opinions of feedlot specialists support the hypothesis that a key determinant of feed quality of sorghum is rate of starch digestion. Although an optimum rate of sorghum starch digestion has not yet been determined, Wester et al. (1992) established that there is a strong positive relationship between in vitro rate of starch digestion and feed/gain ratio ($R^2 = 0.94$). This led us to conclude that improvement of rate of starch digestion in sorghum is a valid breeding objective. Furthermore, Richards et al. (1995) also documented the presence of genetic variation for starch digestion in sorghum, indicating that modification of starch digestion should be possible via plant breeding. However, it has not been possible to select directly for rate of starch digestion in plant breeding programs because of the lack of precision in starch measurement and procedural difficulties and inefficiencies associated with measuring starch digestion rates on the very large numbers of samples generated by a breeding program.

During the development of this 12-h IVDMD procedure for use in breeding for enhanced sorghum grain quality, it was noted that digestion rates of both corn and sorghum were lower than previously reported (Richards et al., 1995; Sniffen et al., 1992). Although the reason for this anomaly has not been determined, containment of the sample within a sealed porous bag in a rotating digestion vessel may reduce contact and exchange of sample with buffered rumen solution as compared with samples suspended directly in buffered rumen solution in traditional in vitro digestion experiments. Regardless of the cause of this anomaly, the effect was similar to stretching the *x*-axis on a graph. Although the time required to reach a particular extent of digestion was roughly doubled compared with the results reported by Richards et al. (1995), our method increased precision, or the ability to detect differences among the same samples.

Adaptation of IVDMD techniques and use of ANKOM fermenters to measure grain digestion at 12 h, establishment of the strong correlation of starch and dry matter digestion at 12 h, and evidence of genetic variation within sorghum for IVDMD after 12-h digestion, demonstrate that the technique can be used by feed grain breeders to improve feed quality. The 12-h IVDMD technique using ANKOM Daisy II fermenters is precise and produces results consistent with known

relationships between corn and sorghum. It is rapid, permitting analysis of approximately 120 samples per fermenter in a 12-h day. Use of sealed filter bags allows scheduling of labor more efficiently, and bulk digestion of samples reduces the opportunity for end-point error associated with very large traditional IVDMD runs.

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