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## Genetic analysis of feed quality and seed weight of sorghum inbred lines and hybrids using analytical methods and NIRS

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### Summary

Eight lines of grain sorghum and their F1 hybrids were evaluated for contents of crude protein (CP), fat (FAT), and starch (STA); protein digestibility (PD); and *in vitro* dry matter disappearance (IVDMD). The effect of seed weight (SW) on these traits and the potential use of near infrared reflectance spectroscopy (NIRS) to predict them also were investigated. The male lines included three normal-seeded lines (TX2737, TX435, and P954063) and two large-seeded lines (PL-1 and Eastin1). The female lines included common U.S. seed parent lines (Wheatland, Redlan, and SA3042). The lines and their hybrids were grown under dryland conditions at Kansas State University experiment fields in Ashland and Belleville, Kansas, in 1999. The experiments were conducted using a randomized complete block design with four replications at each location. The effect of genotype was significant for all measured traits. The male parent lines were highly variable and expressed high levels of genetic variation in combining ability for CP, PD, STA, and SW. The female parents were genetically more uniform; however, significant general combining ability effects were noted for PD and SW. Significant negative correlations were noted between CP and STA and between SW and STA. Significant positive correlations were found between CP and SW and between FAT and IVDMD. Crude protein content was predicted accurately by NIRS. Fat content and IVDMD could not be predicted by NIRS. The NIRS equations based on ground samples were more accurate than those based on whole-seed samples.

### Introduction

Grain sorghum is a major source of dietary protein and energy in the U.S. cattle feedlot industry. However, it traditionally has been considered less valuable than corn because of its highly variable chemical composition and lower feeding value (Hibberd et al., 1982; Rooney & Pflugfelder, 1986). Several studies have reported differences in cattle performance when fed different varieties of sorghum (Hibberd et al., 1979; Streeter et al., 1990, 1993; Wester et al., 1992). Based on the results of these studies, genetic improvement of feed quality of grain sorghum appears possible.

Recently, interest has increased in production of sorghum hybrids with larger seed weight and im-

proved feed quality. Larger seed weight would improve the physical and mechanical handling of grain during processing; however, it could change the chemical composition of grain sorghum, thereby affecting feed quality and digestibility. This possible impact has not been assessed.

Simultaneous genetic improvement for grain size and feed quality requires evaluation of seed weight and the major characteristics of feed: contents of protein, fat, and starch and digestibility. Several studies have investigated the chemical composition of grain sorghum; however, little information is available about inheritance of that composition and digestibility and their associations with feed quality (Hibberd et al., 1979, 1982; Streeter et al., 1993). Knowledge of ge-

netic variability for feed quality characteristics, digestibility, seed weight, and their associations is desirable for designing optimal breeding strategies to improve feed quality.

Several researchers have reported inheritance of protein content in grain sorghum (Liang et al., 1969; Sastry et al., 1986). Breeding programs to increase protein content and digestibility have been initiated (Bramel-Cox et al., 1990). However, protein content is only one component defining the nutritive value of the feed. When formulating diets for growing animals, the greatest cost pressure is the cost per unit energy, most of which is derived from dietary starch and fat. Identifying the best genotypes or combination of genotypes for large seeds, high dietary protein and energy, and high digestibility would be a major step towards integrating seed weight, feed-quality components, and digestibility as objective criteria in genetic improvement of grain and feed quality. Success in these efforts would not only benefit the end user of sorghum but also would enhance the economic competitiveness of sorghum.

Routine determination of chemical composition and digestibility of grain sorghum is of major interest to the feed industry and sorghum breeders. Accurate methods have been developed and are well documented (AOAC, 1990); however, they are relatively complicated, costly, and time consuming and involve use of hazardous chemicals. These difficulties have impeded their application in breeding programs. Therefore, rapid and less hazardous screening methods, such as use of near-infrared reflectance spectroscopy (NIRS), need to be developed and applied to prediction of feed-quality traits and digestibility for grain sorghum. Studies on other cereals have demonstrated that NIRS models of sufficient accuracy for predicting chemical composition can be developed (Williams & Sobering, 1993; Pazdernik et al., 1997; Van Barneveld et al., 1999). The objectives of this study of sorghum were to: (1) provide a genetic assessment of feed-quality traits and *in vitro* digestibility; (2) investigate the impact of seed weight on those traits and digestibility, (3) estimate combining ability for feed-quality traits, digestibility, and seed weight with the aim of identifying the best genotypes or genotype combinations; and (4) investigate the potential use of NIRS in predicting feed-quality traits and digestibility.

## Materials and methods

### *Experiment design and cultural practice*

Five male and three female seed parent lines were intercrossed using a Design-II mating scheme to produce 15 hybrids (Comstock & Robinson, 1952; Hallauer & Miranda, 1988). The males used in this study included three normal-seeded lines (TX 2737, TX 435, and P954063) and two high seed weight lines (PL-1 and Eastin1) and the females were common U.S. seed parent lines (Wheatland, Redlan, and SA3042). The lines and their hybrids (23 entries) were planted and grown under dryland conditions at Kansas State University experiment fields in Ashland and Belleville, KS. The planting dates at the two locations were May 27 and May 28, 1999, respectively. The experiment design consisted of a randomized complete block design with four replications at each location. Entries were grown in single-row plots that were approximately 6 m in length with 0.76 m between rows. Plots were thinned by hand to 129,000 plants ha<sup>-1</sup>.

Plants were harvested by hand at the end of October and mechanically were threshed. Grain samples were oven dried to approximately 10% moisture. Samples of grain from individual plots were cleaned, dried, and ground through a 1 mm screen using a Udy Mill (Udy Corporation, Boulder, Colorado, USA). Ground samples were evaluated for contents of crude protein, crude fat, and crude starch; protein digestibility; and *in vitro* dry matter disappearance as described below.

### *Determination of seed weight (SW)*

Seed weight was determined by measuring 1000 seed weight of grain samples harvested from each plot.

### *Determination of crude protein (CP)*

The CP was quantified based on total nitrogen. Total nitrogen of dry flour samples from each plot (300 mg) was determined using the nitrogen combustion method by Leco CN-2000 (Leco Corporation, 3000 Lakeview, St. Joseph, MI, USA), an instrument consisting of a combustion, collector, and measuring system. The instrument was calibrated using an apple leaf, a standard reference material No. 1515 with a known amount of nitrogen and approved by NIST. A control sample of apple leaf with a weight equivalent to that of the sorghum sample was included in each of the runs. The CP in each sample was estimated from total nitrogen

using a numerical conversion factor of 6.25 (Hibberd et al., 1982; Sastry et al. 1986; Wester et al., 1992; Buffo et al., 1998). The resulting value was multiplied by a 1000 to obtain CP ( $\text{g kg}^{-1}$ ).

#### *Determination of crude fat (FAT) and crude starch (STA)*

The FAT was extracted and quantified using the ether extraction method No. 920.39 (AOAC, 1990). The STA was evaluated similarly using method No. 979.10 (AOAC, 1990).

#### *In vitro protein digestibility (PD)*

A slightly modified pepsin (Sigma P-7000; activity 120 units/mg of protein) digestion procedure was used to quantify PD (Mertz et al., 1984; Oria et al., 1995). Ground flour samples (375 mg) were incubated in the buffered pepsin enzyme solution at 37 °C for 2 hours in a shaking water bath. Protein digestibility ( $\text{g kg}^{-1}$ ) was calculated by subtracting undigested protein from total protein and dividing by the total protein.

#### *In vitro dry matter disappearance (IVDMD)*

The IVDMD was quantified using the procedures developed by Pedersen et al. (2000). Two 0.5 g ground samples from each plot at each location were sealed in ANKOM F57 filter bags (95% of pores <30 microns), dried at 60 °C for 24 hours, and weighed to determine sample dry weight. Samples were placed in vessels containing buffer solution and rumen inoculum, the vessels were purged with CO<sub>2</sub>, and lids with gas relief valves were placed on the vessels. The samples then were placed into an ANKOM Rumen Fermentors Model No. Daisy II and incubated continuously at 39 °C. After 12 hours of digestion, the vessels were removed, and IVDMD ( $\text{g kg}^{-1}$ ) was calculated as the difference between the preweighed sample and residue sample divided by the initial sample weight.

#### *Statistical analyses*

Analyses of variance (ANOVA) were conducted across locations for all traits using the PROC GLM procedure of the SAS Statistics package (SAS, 1990). Each of the six traits was computed on a per plot basis. The preliminary analysis involved tests for the effects of location, entry, and location  $\times$  entry interaction. Expected mean squares for each source of variation

were computed and used to apply the appropriate F-test according to standard procedures (Hallauer and Miranda, 1988). Entries were partitioned into variation within parents and within hybrids. The parent sum of squares was subdivided into males and females. Hybrids sum of squares were partitioned into male, female, and male  $\times$  female interaction. Tests of significance for males, females, male  $\times$  female, female  $\times$  location, male  $\times$  location, male  $\times$  female  $\times$  location interactions for all traits were made by testing the mean squares with their respective location interaction mean squares. Phenotypic correlations among the feed-quality traits, seed weight, and *in vitro* digestibility also were computed. The impact of seed weight was determined by relating the phenotypic performance of the inbreds and hybrids for feed-quality traits and digestibility to seed weight. General combining ability (GCA) effect for each parent was computed using the methods of Beil & Atkins (1967). The significance of GCA effects was determined using the least square significance difference as proposed by Cox & Frey (1984). The male  $\times$  female interaction was used to test the importance of specific combining ability (SCA).

#### *NIRS analysis*

A NIRS Model DA7000 Perten (Perten Instruments, Inc. Springfield Illinois, USA) diode array spectrometer was used for NIRS analysis. Ground samples were placed in a mini petri dish (60  $\times$  15 mm), and the system was allowed to automatically collect the spectra, resulting in 15 sequentially numbered spectra per sample. The NIRS system then was recalibrated to measure whole-seed samples in a similar way.

All data were analyzed using partial-least squares (PLS) regression, which is a spectra decomposition technique, performed by a module of GRAMS/32 software (Galactic Industries, Salem, NH). Martens & Naes (1989) provide a complete description of PLS. All spectra were mean centered prior to analysis. The PLS method was chosen over the principal components technique because it takes advantage of concentration information. The optimum number of PLS factors was determined by the point at which no further improvement was made to the standard error of cross validation (SECV) of the calibration. The SECV provides the best estimate of calibration accuracy obtained from a single set of samples sourced on the same occasion (Van Barneveld et al., 1999). Means and standard deviations (SD) and correlations between

Table 1. Mean squares from the analysis of variance for feed-quality traits and seed weight in grain sorghum hybrids tested over two locations

Source variation <sup>a</sup>	df	Trait Mean Squares <sup>b</sup>					
		CP <sup>c</sup>	PD	IVDMD	FAT	STA	SW
Location (L)	1	3.94	2334.51	1.27	0.06	9.31	10.00
Replication/(L)	6	2.08**	248.98**	36.89**	0.39**	10.98**	7.50**
Entry	22	14.36**	127.03**	12.53	0.21**	23.15**	605.04**
Inbred (I)	7	7.82**	253.93**	16.85	0.21*	11.96	904.82**
I vs H	1	213.56*	2.94	6.29	0.72	325.35*	43.57
Hybrid (H)	14	3.40**	72.45**	10.82	0.18	7.16*	495.26**
Male (M)	4	10.36**	121.32**	19.66	0.33	19.61*	1715.83**
Female (F)	2	0.56	20.51	3.34	0.05	2.75	19.07**
M × F	8	0.63	60.99*	8.27	0.13	2.04	4.02
L × Entry	22	0.80**	26.28	8.98	0.07	4.25*	4.82**
L × I	7	1.03	28.63	9.36	0.05	8.03	7.12*
L × I vs H	1	1.59	226.19	19.43	0.18	0.68	27.10
L × H	14	0.63	10.83	8.04	0.07	2.62	2.08
L × M	4	0.24	4.00	6.12	0.10	1.19	1.47
L × F	2	10.99**	3.41	8.51	0.01	10.78*	0.03
L × M × F	8	0.49	16.09	8.89	0.08	1.31	2.89*
Error a <sup>1</sup>	84	0.26	28.40	7.04	0.09	2.48	1.17
Error b <sup>2</sup>	132	0.26	26.28	7.04	0.09	2.60	1.69

<sup>a</sup> a<sup>1</sup> = Error term for design II analysis, b<sup>2</sup> = General error term.

<sup>b</sup> CP = Crude protein, PD = Protein digestibility, IVDMD = *In vitro* dry matter disappearance, FAT = Fat, STA = Starch, and SW = Seed weight.

<sup>c</sup> \*, \*\* Significant at  $p < 0.05$  and  $0.01$  respectively.

actual and NIRS predicted values,  $r_{AP}$ , from spectra data were computed. The RPD value was computed as  $RPD = SD/SECV$ , where SD is the standard deviation of actual values from the original data. The results were compared on the basis of the coefficient of determination,  $r^2$ , SECV, RPD, and  $r_{AP}$ . The RPD statistic was used to provide a basis for standardizing the standard error of prediction (SEP). The SECV statistic was used to select the best calibration equation. All analyses were carried out across locations including all data. However, separate calibration and analysis were conducted for ground and whole-seed samples.

## Results

The analyses of variance for CP, PD, IVDMD, FAT, STA, and SW are reported in Table 1. The effect of entry was highly significant for all the measured traits, except for IVDMD. The entry source of variation was partitioned to evaluate the significance of variation among different subgroups of entries (Table 1). Significant differences among inbred lines were noted for all traits except for IVDMD and STA. Significant dif-

ferences among the hybrids were noted for CP, PD, STA and SW. The difference between inbred vs. hybrid performance was significant only for CP and STA. The hybrid source of variation was partitioned to determine the importance of the male and female parents in expression of each trait (Table 1). The male parent effect was highly significant for CP, PD, STA and SW, whereas the female effect was significant only for SW. Except for PD, no significant effect of the male × female interaction was noted. The main effect of location on the measured characteristics was not significant; however, a few of the location interactions were significant (Table 1). Only CP and SW showed highly significant values for the location × entry interaction. No significant male × location interactions were detected, but significant location × female interaction were noted for CP and STA. The location × female × male interaction was not significant, except for SW. The effects of location × hybrid, location × inbred, and location × inbred vs hybrid were not significant.

The phenotypic correlations among the measured traits are presented in Table 2. A highly significant

Table 2. Phenotypic correlations between feed quality traits and seed weight

Traits <sup>a</sup>	CP <sup>b</sup>	PD	IVDMD	FAT	STA
PD	-0.01				
IVDMD	-0.14	-0.11			
FAT	0.03	0.00	0.24*		
STA	-0.61**	0.03	-0.05	-0.06	
SW	0.25**	-0.02	0.09	0.09*	-0.22*

<sup>a</sup> CP = Crude protein, PD = Protein digestibility, IVDMD = *In vitro* dry matter disappearance, FAT = Fat, STA = Starch, and SW = Seed weight.

<sup>b</sup> \*, \*\* Significant at  $p < 0.05$  and  $0.01$  respectively.

negative correlation was noted between CP and STA (-0.61). Significant positive and negative correlations also were noted between CP and SW (0.25), FAT and IVDMD (0.24), and SW and STA (-0.22). Other correlations were low and not significant, ranging from -0.14 to 0.09.

Least square mean values for CP, PD, IVDMD, FAT, STA, and SW for inbreds and hybrids are presented in Table 3. Considerable variation in phenotypic performance was noted among parent lines and hybrids. The values of CP for hybrids varied from 106 to 130 g kg<sup>-1</sup> and were generally lower than those from the parent lines. The male parent lines varied significantly in SW (Table 3). PL1 and Eastin1 were the largest-seeded entries with seed weights that were nearly twice those of the other parent lines. The values of SW for hybrids ranged from as low as 26.9 to as high as 48.8 g 1000 seed<sup>-1</sup>. The SW of hybrids was generally intermediate or smaller than the mid-parent average; however, SW in PL1 hybrids appeared to be inherited as an incompletely dominant trait. Little variation in FAT occurred among the hybrids and inbreds (31.6 to 36.9 g kg<sup>-1</sup>). The values of FAT for hybrids were not significantly different from those from the parental lines. Variations in STA among inbreds (583 to 623 g kg<sup>-1</sup>) and hybrids (613 to 645 g kg<sup>-1</sup>) were small. The values of PD were more variable among the parental lines (323 to 502 g kg<sup>-1</sup>) than among the hybrids (330 to 450 g kg<sup>-1</sup>). TX 2737, a standard U.S. pollinator line, had the highest PD among inbred parents, and its hybrids also tended to have high levels. Very little variation in IVDMD was noted among inbreds or hybrids. The values of IVDMD ranged from 362 to 404 g kg<sup>-1</sup> for the inbred lines and from 375 to 414 g kg<sup>-1</sup> for the hybrids.

The values of combining ability for feed quality and seed weight are presented in Table 4. The male

parent lines were highly variable and expressed high levels of genetic variation in combining ability for CP, PD, STA, and SW. The female parents were genetically more uniform; however, significant GCA effects were noted for PD and SW.

The results from NIRS calibration equations for feed-quality traits and *in vitro* digestibility are reported in Table 5. The table includes the means of predicted values and correlations between actual and predicted values for CP, PD, IVDMD, FAT, and STA. Crude protein had the largest coefficients of determination for ground and whole-seed samples,  $r^2 = 0.82$  and  $r^2 = 0.64$ , respectively. It also had the largest RPD values for ground samples (2.34) and whole-seed samples (1.65) and the highest  $r_{AP}$  values for both ground and whole-seed samples, followed by STA. Fat content and IVDMD had the smallest values of  $r^2$ , RPD, and  $r_{AP}$  for ground and whole seed samples. In all the predictions, calibration equations based on ground samples more accurate than those based on whole-seed samples.

## Discussion

The values for feed-quality traits and digestibility in this study indicated considerable genetic variation among genotypes but were within the range of expected values. The values for protein content found in this study were consistent with those reported in the literature, which indicated ranges from 103 to 165 g kg<sup>-1</sup> for the inbred lines and from 89 to 155 g kg<sup>-1</sup> for the hybrids (Streeter et al., 1990; Hibberd et al., 1980, 1982, 1983; Sastry et al., 1986; Wester et al., 1992; Streeter et al., 1993). However, the values reported here are larger than the average of 89 g kg<sup>-1</sup> from nine regions within Kansas reported by the Kansas Grain Sorghum Commission (1997). This difference may reflect the more marginal sorghum production conditions, including reduced fertility, commonly encountered under farm production conditions. The values of STA ranged from 583 to 623 g kg<sup>-1</sup> for inbred lines and from 613 to 645 g kg<sup>-1</sup> for the hybrids. This is consistent with the values of 618 to 705 g kg<sup>-1</sup> reported by Hibberd et al. (1980). The values reported for the hybrids, however, are lower than those of 726 to 799 g kg<sup>-1</sup> reported by Streeter et al. (1993) and those reported by Wester et al. (1992) in a study involving 48 hybrids differing in starch and protein compositions. The fat content reported in this study ranged from 31.7 to 36.3 g kg<sup>-1</sup> among the inbred lines and from

Table 3. Least square mean values for inbred lines and hybrids for feed-quality traits and seed weight

Genotypes <sup>a</sup>	Trait <sup>b</sup>					
	CP g kg <sup>-1</sup>	PD g kg <sup>-1</sup>	IVDMD g kg <sup>-1</sup>	FAT g kg <sup>-1</sup>	STA g kg <sup>-1</sup>	SW g 1000 <sup>-1</sup> seed
Males						
Eastin1	138	439	393	32.1	605	47.2
P954063	134	363	373	31.9	623	29.4
PL1	136	403	401	32.9	605	55.2
TX2737	127	502	404	31.7	595	26.2
TX435	129	382	380	32.6	615	28.2
Females						
Redlan	153	323	362	33.6	605	29.6
SA3042	153	346	384	34.9	583	28.7
Wheatland	142	382	395	36.3	595	31.0
Hybrids						
Redlan × Eastin1	120	409	414	35.0	629	36.4
SA3042x Eastin1	122	408	399	36.0	628	35.0
Wheatland × Eastin1	122	397	389	35.0	623	36.9
Redlan × P954063	111	384	379	33.7	642	27.3
SA3042 × P954063	114	423	377	33.7	631	28.2
Wheatland × P954063	111	382	375	31.6	642	29.2
Redlan × PL1	130	363	394	36.6	613	46.2
SA3042 × PL1	122	361	378	36.9	620	46.5
Wheatland × PL1	124	376	389	34.7	618	48.8
Redlan × TX2737	114	402	392	33.0	641	27.8
SA3042 × TX2737	113	426	380	34.0	628	28.4
Wheatland × TX2737	106	450	405	36.4	635	28.0
Redlan × TX435	111	408	388	35.1	645	27.6
SA3042 × TX435	114	330	405	33.7	636	26.9
Wheatland × TX435	111	410	394	33.1	634	28.3
SE	1.8	18.1	9.4	1.1	5.7	0.46
LSD (0.05)	5.1	51.0	26.2	3.0	16.0	1.28

<sup>a</sup> SE and LSD are the standard error and the least significance difference, respectively, based on the 23 entries.

<sup>b</sup> CP = Crude protein, PD = Protein digestibility, IVDMD = *In vitro* dry matter disappearance, FAT = Fat, STA = Starch, and SW = Seed weight.

31.6 to 36.4 g kg<sup>-1</sup> among the hybrids. These were somewhat higher than those found in some previous studies (Saba et al., 1964; Keating et al., 1965; Schake et al., 1976) but were near the average of 31 g kg<sup>-1</sup> reported by the Kansas Grain Sorghum Commission (1997). The high level of fat observed here would be beneficial to the feed industry, because fat is a major source of energy. The differences between the values of the feed traits in this study and those reported in the literature are understandable given the differences in genetic materials used.

Most feed quality characteristics measured in this study were inherited as genetically additive traits, and mid-parent averages were good indicators of relative

hybrid performance. However, some traits were more dominant or recessive in nature depending on the genetic background. For example, SW was inherited as additive or recessive in some genetic backgrounds, but was more dominant in hybrids derived from PL1. Given this variation in additive and dominant behavior, careful selection of parent is important when identifying germplasm sources for genetic enhancement of a given feed quality trait. Plant breeders working on hybrid crops prefer to use germplasm sources having either dominant or additive genetic effects that can be incorporated into genetic backgrounds of either pollinator parent or seed parent lines. The incorporation of these types of genes into a single parent line requires

Table 4. General combining ability for feed-quality traits and seed weight

Lines	Trait <sup>a</sup>					
	CP <sup>b</sup> g kg <sup>-1</sup>	PD g kg <sup>-1</sup>	IVDMD g kg <sup>-1</sup>	FAT g kg <sup>-1</sup>	STA g kg <sup>-1</sup>	SW g 1000 <sup>-1</sup>
Males						
Eastin1	5.2**	9.5**	0.010	8.0	-4.5**	2.65**
P954063	-4.3**	1.0	-0.014	-1.5	7.4**	-5.21**
PL1	8.9**	-28.8**	-0.003	1.5	-13.7**	13.75**
TX2737	-5.5**	30.8**	0.002	-0.1	3.5**	-5.38**
TX435	-4.3**	-12.5**	0.005	-0.6	7.3**	-5.81**
SE <sub>GCA</sub>	0.9	3.7	0.004	0.6	2.0	0.22
Females						
Redlan	0.7	-2.1	0.003	0.1	2.9	-0.37 **
SA3042	0.7	-5.9	-0.003	0.3	-2.3	-0.43 **
Wheatland	-1.4	8.0	0	-0.4	-0.6	0.80 **
SE <sub>GCA</sub>	3.3	4.4	0.007	0.2	7.7	0.04
Grand mean	116.3	395	390	34.6	631.1	33.43

<sup>a</sup> CP = Crude protein, PD = Protein digestibility, IVDMD = *In vitro* dry matter disappearance, FAT = Fat, STA = Starch, and SW = Seed weight.

<sup>b</sup> \*, \*\* Significant at  $p < 0.05$  and  $0.01$  respectively.

Table 5. Near infrared spectroscopy (NIRS) calibration equation statistics for feed-quality traits and digestibility using 184 sorghum grain samples that were analyzed as ground or whole seeds

Trait <sup>a</sup>	Mean	Ground sample				Seed sample			
		R <sup>2</sup>	SECV	RPD	r <sub>AP</sub>	R <sup>2</sup>	SECV	RPD	r <sub>AP</sub>
CP (g kg <sup>-1</sup> )	124	0.82	0.62	2.34	0.91	0.64	0.88	1.65	0.80
PD (g kg <sup>-1</sup> )	394	0.41	5.87	1.30	0.64	0.19	6.96	1.10	0.44
IVDMD (g kg <sup>-1</sup> )	389	0.02	2.94	1.01	0.15	0.00	3.0	0.99	-0.06
FAT (g kg <sup>-1</sup> )	34.1	0.0	0.34	0.98	0.24	0.06	0.33	1.00	0.24
STA (g kg <sup>-1</sup> )	621	0.45	1.79	1.32	0.66	0.35	1.91	1.23	0.59

<sup>a</sup> CP = Crude protein, PD = Protein digestibility, IVDMD = *In vitro* dry matter disappearance, FAT = Fat, and STA = Starch.

less work and reduces concerns over loss of genetic diversity among germplasm pools of male and female parents.

Small-seeded hybrids generally produced lower CP and higher STA values than larger-seeded lines and hybrids. This is consistent with previous reports (Sepel & Sepel, 1970; Sastry et al., 1986). The reason for higher protein content and lower starch content in large-seeded lines and their hybrids was not clear. From the animal nutrition standpoint the negative correlation between protein content and starch is undesirable. The moderate negative correlation between CP and STA observed in this study tend to indicate that an increase in protein content may lead to a quantitative reduction in starch or dietary energy. However, the negative correlations between protein and starch

and between seed weight and starch are not so high as to prevent simultaneous genetic improvement of the three traits. Moreover, a deficiency in dietary energy may be overcome by the amount of fat in the feed, because fat seemed to be stable across lines and hybrids.

The low positive correlation between seed weight and protein content and the low negative correlation between seed weight and starch content suggest that increased seed weight would not seriously affect the feed-quality characteristics; however, simultaneous increase of seed weight and improvement in feed quality would be ideal. The higher CP and SW and only slightly lower values of STA observed in hybrids derived from PL1 and Eastin1 indicate that these two lines could be used as male parents in developing



large-seeded hybrids with increased protein content without seriously compromising dietary energy.

The values of protein digestibility in this study were 323 to 502 g kg<sup>-1</sup> for inbred lines and 361 to 450 g kg<sup>-1</sup> for hybrids. These were somewhat lower than the values of 658 to 894 g kg<sup>-1</sup> reported in the literature (Hamaker et al., 1986, 1987; Weaver et al., 1998). The differences may be explained by differences in particle size of grain samples as well as the genetic diversity of the lines and hybrids evaluated in this study. The grain samples used in this study were ground through a 1-mm screen, whereas samples in the previous studies were ground through a 0.4-mm screen. The particle size of the ground sorghum sample is important in the pepsin test. Reducing the particle size to 0.4 mm increases the surface area of the sample, thereby increasing enzyme activity and digestibility. Axtell et al. (1981) reported a value of 343 g kg<sup>-1</sup> for a P-721 grain sorghum sample ground through 1mm screen using the Udy mill compared with 467 g kg<sup>-1</sup> after that sample was reground through 0.4mm screen Hobart coffee grinder. In this study, protein digestibility was used to estimate digestion in monogastrics (humans and swine). However, it worth noting that digestibility differs with respect to the animals. Although finely ground samples are important in protein digestibility and may be justifiable for monogastric animals, grinds of less than 1 mm may have little effect on digestion rate in ruminants and are subject to mechanical losses and escape during bag rinsing. Loss of sample during bag rinsing may lead to biased or unreliable IVDMD values. In this study, samples ground through 1-mm screen were used to ensure a degree of uniformity and to make the outcomes from each analysis comparable.

The values of IVDMD based on a 12-hour digestion trial for inbred lines ranged from 362 to 404 g kg<sup>-1</sup> and from 375 to 414 g kg<sup>-1</sup> for hybrids. These values tended to fall at the lower end of the range from 292 to 616 g kg<sup>-1</sup> reported by Hibberd et al. (1980, 1982) for 24-hour digestion trials, and were significantly higher than the values of 81 to 314 g kg<sup>-1</sup> reported by Hibbert et al. (1982) for a 6-hour digestion trial. These differences are understandable given that digestibility is a function of incubation period.

No significant differences in IVDMD were detected among lines or hybrids. The poor correlations between IVDMD and PD and other feed-quality traits suggest that determining the inheritance of digestibility would be difficult. They may be due partly to diversity in the genetic material used and the accu-

acy of obtaining measurements of digestibility. The low correlation between the PD and IVDMD is understandable, because protein digestion based on pepsin activity is different from rumen-based assays. The correlations of fat with other components feed-quality traits and SW were close to zero. This indicates that concurrent improvements in CP, STA, and SW would not affect the amount of fat.

Significant positive and negative values of GCA were found for seed weight, digestibility, and feed-quality traits among the male parents and for digestibility and SW among the female lines. The male parent lines were genetically more diverse than female parents, resulting in more significant GCA effects among males. The females used in this study represent standard U.S. seed parent lines and were genetically more uniform than the male parent lines. Given the limited degree of genetic variation among female parents, few of these GCA effects were significant. In addition, these effects were tested for significance using the location  $\times$  female interaction mean square, which inflated the standard error. The male  $\times$  female interaction was not significant for most traits, indicating that specific combining ability was not a major component of the genetic variation observed among hybrids.

Preliminary studies were carried out to determine if NIRS could be used to predict the three major feed-quality traits and digestibility. The high values of coefficient of determination and RPD for CP observed in this study indicate that NIRS could be used to predict protein content in grain sorghum and are consistent with previous results for other cereals (Williams et al., 1984; Williams & Sobering, 1993). Although the  $r^2$  values for PD and STA were somewhat low, the RPD values of greater than 1.00 and the moderate  $r_{AP}$  values suggest that NIRS could be used to screen for these traits. The low  $r^2$ , RPD, and  $r_{AP}$  values for IVDMD and FAT indicate that these traits were not predictable using NIRS. Statistically, an RPD value of 1 or below means that the SECV value is equal to or below the standard deviation of the actual measurement, indicating that such a trait is not predictable using NIRS (Williams & Sobering, 1993). It is not clear from this study why FAT and IVDMD produced such low values. Poor prediction would be expected in a sample set that had little variation for these traits; however, the biochemical complexity of these traits also could play a role.

The actual mean NIRS values (not presented) and the predicted mean values were identical, whereas the SDs for actual NIRs values were larger than the

SDs for predicted values (not presented) for individual traits. This indicates that although the means of individual traits could be predicted accurately using NIRS, the same could not be said for individual samples. Predicted values from equations based on ground samples were higher than those from equations based on whole seed samples. Pazdernik et al. (1997) used NIRS to analyze amino acid and fatty acid compositions in soybean seed and also found that calibration equations based on ground samples were more accurate than those based on seed samples. The lower values for the calibration equations from seed samples may be attributed to diversity of surface area and size of the materials used. Assuming that grinding costs are low, use of ground samples is recommended; otherwise initial screening could be done with seed samples and final screening with ground samples or chemical analysis.

The results from this study show that genetic variation for feed-quality traits, protein digestibility, and seed weight in grain sorghum is sufficient to effectively breed for improved feed quality and increased seed weight. The principal impact of this research is in clarifying the effect of increasing seed weight on feed-quality traits and digestibility. Large-seeded sorghum hybrids with high feed quality can be developed when clear economic incentives exist for doing so. However, one of the main factors limiting this effort will be the cost associated with characterizing large numbers of breeding lines and experimental hybrids. Determining feed quality of grain sorghum is a costly, laborious, slow, and hazardous task. Preliminary results in this study indicate that sorghum breeders could use NIRS technology for screening of samples varying in their protein and energy contents. This technique is rapid, portable, and less costly than other existing methodologies. However, more research is needed to evaluate the use of NIRS in grain sorghum.

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