DRY MATTER DIGESTIBILITY IN PURPLE TESTA SORGHUMS[1]

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The feeding efficiency of sorghum grain is highly variable. This variability is associated with its market classification (10, 17), endosperm texture and starch type (8), protein composition and content (24), and environmental effects (13). Sorghums of the market class brown sorghum are grown in areas of high bird depredation for their bird resistance, but their grain has been found to have reduced digestibility in animals (29).

The market class of brown sorghum has pigmented testae and may have pericarps containing brown pigment (19). Sorghums whose pericarps contain brown pigment have a testa. Brown sorghums have become associated with bird resistance due to the presence of astringent polyphenolic compounds in the immature grain from which the pigment is developed. The astringent fraction of the polyphenolic compounds is referred to as "condensed tannins," and their activity is at the highest level in the immature stage of grain development. This activity is reduced in the mature grain (3). Bird repellent properties result from an astringent action caused by

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these compounds complexing with salivary and mucosal proteins (4).

Chang and Fuller (7) reported tannin content of brown sorghums to range from 1.3 to 2 percent. These tannins have been described as having a molecular weight of between 500 and 3000 which enables them to form effective cross links with proteins and other macromolecules (27).

Figure 1 is a schematic transverse section of a feterita seed (25). This transverse section shows the pericarp, testa, and endosperm. The pericarp or the fused pericarp and testa make up the "seed coat" of a sorghum caryopsis. The pericarp and testa constitute about 6 percent of the grain (14).

Pigments found in the pericarp and testa are polyphenolic in composition. Yellow, pink, and red are the nonbrown pericarp pigments. Blakely et al. (2) also found pigments in the epicarp and in cross and tube cells of the pericarp; white was sorghum without pigment. They also found sorghums with the dominant testa genes to have a pigmented testa and those with both dominant testa and dominant spreader genes to have brown pigments in the epicarp and the cross and tube cells. The various shades of brown (testa present) found in the pericarp are due to an independent development of white, yellow, pink, or red pigment in addition to the brown (30).

Swanson (26) found that the testae may vary in development. In some

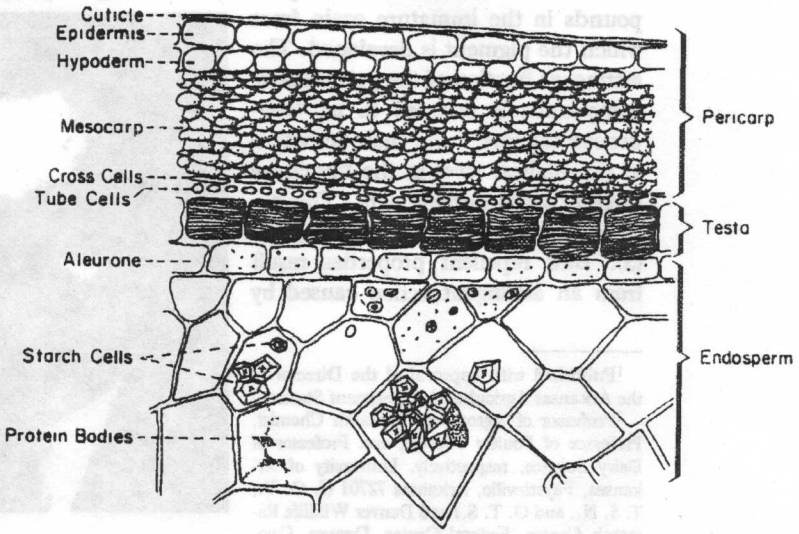

Figure 1. A schematic transverse section of a portion of a feterita seed. [Swanson (1926)]

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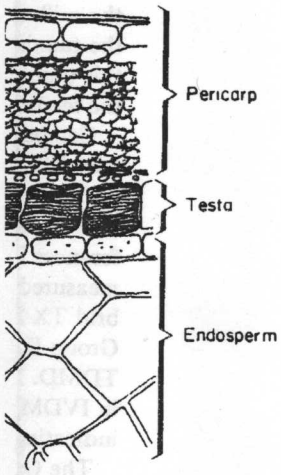

eterita seed. [Swanson (1926)]

varieties it may be strongly developed, in others much reduced or represented by a trace, and in others absent. Sanders (21) reported that the testa always is initially present in the ovary, but in many varieties it is absorbed and thus absent before the kernels attain full development. He found pigment in the cells of the testa of hegari before pollination. He also found that more pigments were laid down as the grain matured. The dominant S gene (spreader) allows brown pigment (tannins) to spread to the pericarp when a testa is present (25).

Morall et al. (18) found in two brown sorghum hybrids small tannin-containing vesicles which appeared at the inner integument cells just after fertilization. At the milk stage of grain development, tannins were rapidly being deposited along the periphery of the central vacuole. The tannins developed until there was little evidence of cell structure and the testa consisted of a continuous layer of tannin. The tannins extended from the testa through the cross and tube cells into the mesocarp. Once deposited, the tannins did not appear to decrease as the grain matured.

Differences in feeding efficiency have been reported in the brown sorghums (9, 13, 23). Cummings and Axtell (9) classified sorghums into three groups based on tannin analyses and presence or absence of a testa. Tannin analyses were based on the vanillin (V) method and the modified vanillin (MV) method (5, 16). Group I had low catechin equivalent (CE) values in V and MV tests but did not contain a testa. Group II had a low CE value on V and a high CE value on MV tests and contained a testa. Group III had high CE values on both V and MV tests and contained a testa. In a rat feeding trial (9) they found the presence of a testa to be an inaccurate indicator of nutritional quality since rats fed rations prepared from Group II bulked samples of grain with testae showed growth rates equivalent to those of rats fed diets from Group I.

Bullard et al. (4) suggested five features that should be considered in the loss in tannin activity in Group II sorghums during ripening:

1) the polymerization process,
2) the skeletal structure of the tannin molecule,
3) extractable grain components,
4) nonextractable grain tissue components, and
5) structure of the testa.

Peroxidase and polyphenol oxidase (PPO) are capable of oxidizing phenolic compounds. Glennie (11) analyzed a Group III sorghum at various stages of preharvest development for tannin and polyphenol oxidizing en-

zymes. Tannins appeared first at the milk stage, reached a maximum at the hard dough stage, and thereafter declined by 25 percent. Polyphenol oxidase (PPO) and peroxidase activities were detected during the flowering stage. PPO activity declined rapidly as the grain began to develop while the decline of peroxidase was slower. Neither activity could be detected in mature grain. York et al. (31) reported finding in the mature grain of a Group I sorghum a PPO activity of 36, in a Group II an activity of 22, and in a Group III an activity of 7.

The Group II sorghum had a purple testa, and its feeding efficiency was equivalent to that of Group I sorghum (31). Casady (6) reported two testa colors, one brown and the other purple. In an inheritance study, he found brown dominant to purple.

The objective of this research was to study the effect of the tannin produced by the purple testa sorghums on dry matter digestibility of the grain.

MATERIAL AND METHODS

A group of sorghum hybrids and lines were grown on the Agronomy Farm of the Arkansas Agricultural Experiment Station at Fayetteville in 1980. From this group, grains from 10 sorghums were chosen for chemical and dry matter digestibility studies. In this group of 10, there were two Group I sorghums, five Group II sorghums, and three Group III sorghums:

TABLE 1—Grain Descriptions of the Sorghums Used in this Study, 1980.

Sorghum	Testa Color	Testa Genes	Pericarp Color
Group I Sorghums			
TX 399	abs.	Tp Tp	P
TX 2761	abs.	Tp Tp	P
Group II Sorghums			
AR 3009	purple	tp tp	W
N.K. X3229	purple	tp tp	P
TAM 2566	purple	tp tp	W-B
TX 2761 X TAM 2566	purple	tp tp	R-B
TX 6649[1]	purple	tp tp	W
Group III Sorghums			
AR 3003	brown	Tp Tp	P-B
DeKalb BR54	brown	Tp Tp	P-B
TX 399 X TAM 2566	brown	Tp tp	P-B

[1]Cultivar S. A. 6649-7-2, Double Dwarf Feterita.

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ta ies	Pericarp Color
Tp	P
Tp	P
tp	W
tp	P
tp	W-B
tp	R-B
tp	W
Tp	P-B
Tp	P-B
tp	P-B

Tannin activity of the mature grain of these sorghums was measured by protein precipitation (PP) and an improved modified-vanillin assay (MV) using partially purified BR 54 sorghum tannin as standard (3, 12, 20).

Polyphenol oxidase activity (PPO) was also measured in the mature grain. It was measured using the standard procedure with a phosphate buffer, pH 5.8, and pyrogallol substrate. The Biuret methods of protein determination were used to measure PPO activity. Percentage of protein was obtained from each sample using the micro-Kjeldahl procedure.

Dry matter digestibility was obtained from each sample by two methods. These sorghums were fed to 24 4-week old chicks and their dry matter digestibility was measured by the true dry matter digestion method (TDMD) of Kirby et al. (15). *In vitro* dry matter digestibility (IVDMD) was determined for the 10 sorghums in an artificial rumen using the Tilley-Terry technique (28) as modified by Barnes (1).

Correlations among the data for PP, MV, PPO, TDMD, and IVDMD were made to determine the relationship of the methods in determining tannin activity (22).

RESULTS AND DISCUSSION

An ideal bird-resistant sorghum grain would be highly astringent during the milk and dough stages of grain development with a great reduction in the tannin fraction of the polyphenols in the mature grain. The activity of this tannin fraction must be reduced to a level that does not interfere with the feeding efficiency of the grain.

Comparisons of the Group I, Group II, and Group III sorghums in protein, PP, MV, PPO, TDMD, and IVDMD are given in Table 1. Differences in percentage of protein were found within groups rather than among groups.

Groups I and II were lower in PP than was Group III. There were no differences in PP for the sorghums of Groups I and II. The tannin activity as measured by MV was higher in Group III than in Groups I and II. The hybrid TX 2761 X TAM 2566 was higher in MV than the other sorghums in Group II. This hybrid acted as a Group III in IVDMD and as a Group I in TDMD. Something in the grain of this sorghum is suppressing digestibility in IVDMD but not in TDMD. The increase in MV for this hybrid may be indicating the IVDMD suppressant.

The Group III hybrid TX 399 X TAM 2566 is heterozygous for a brown testa. It has a PP and MV lower than the other Group III sorghums and higher than the Group I and II. This is reflected in the IVDMD and TDMD.

The heterozygous condition of the brown testa X purple testa resulted in a brown testa hybrid with a lower amount of tannin.

The PPO indicates a general wide range in activity (Table 2). The analyses for this activity were made in July of 1982. The analyses of the same grain of TX 399, TAM 2566, and AR 3003 were also made in January of 1981, at the same time the TDMD and IVDMD were made. The PPO activities of the first analyses were 37 for TX 399, 22 for TAM 2566, and 7 for AR 3003. Analyses of 1981 produced grain immediately after harvest gave PPO activities of 31 for TX 399, 27 for TAM 2566, and 0 for AR 3003. These analyses indicate that aging of the grain plays a role in PPO activity. The greatest change due to aging was in TX 399, a Group I sorghum. Glennie (11) did not find PPO activity at maturity in a Group III sorghum.

The TDMD does not indicate a wide range in digestibility in the sorghums of the different groups, whereas the IVDMD does. Poultry has been able to digest the higher tannin sorghums more efficiently than the other monogastric and ruminant animals. Sorghum with poor IVDMD digestibility are found in each group. The Group III sorghums and one Group II sorghum have the lowest IVDMD digestibilities.

TABLE 2—Comparisons of Group I, II, and III Sorghums by Percentage of Protein, Protein Precipitation (PP), Modified Vanillin (MV), Polyphenol Oxidase Activity (PPO), True Digestion of Dry Matter by Chicks (TDMD), and IN VITRO Dry Matter Digestibility (IVDMD) Using Mature Grain, 1980.

Sorghum	Protein	PP[1]	MV[1]	PPO[2]	TDMD[3]	IVDMD[3]
Group I Sorghums						
TX 399	12.5	0.04d	0.25e	16ab	89.4bc	75.7b
TX 2761	13.0	0.03d	0.20e	20a	92.3abc	68.0d
Group II Sorghums						
AR 3009	13.2	0.02d	0.21e	20a	89.9bc	80.2a
N.K. X3229	11.2	0.03d	0 25e	16bc	93.7abc	73.4bc
TAM 2566	13.1	0.02d	0.20e	21a	92.5abc	70.9ed
TX 2761 X TAM 2566	13.2	0.04d	0.37d	15bc	95.6ab	56.8f
TX 6649	11.6	0.05d	0.26e	20a	97.0a	82.8a
Group III Sorghums						
AR 3003	11.3	0.75a	3.38a	6d	87.1c	57.3f
DeKalb BR 54	10.4	0.56b	1 69b	12c	89 1cb	56.4f
TX 399 X TAM 2566	11.8	0.33c	1.05c	18ab	92.8abc	63.6e

[1]DeKalb BR 54 equivalents.
[2]Units/mg protein/minute.
[3]Percentage of corn.
[4]Means followed by the same letter are not significantly different at the 0.05 level.

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	PPO[2]	TDMD[3]	IVDMD[3]
	16ab	89.4bc	75.7b
	20a	92.3abc	68.0d
	20a	89.9bc	80.2a
	16bc	93.7abc	73.4bc
	21a	92.5abc	70.9ed
	15bc	95.6ab	56.8f
	20a	97.0a	82.8a
	6d	87.1c	57.3f
	12c	89.1cb	56.4f
	18ab	92.8abc	63.6e

fferent at the 0.05 level.

Neither tannin activities nor animal digestibilities follow a set pattern when working with the brown sorghums. Unexpected results from data by the biochemists or nutritionists have causes that should be investigated. Cummings and Axtell (9) found in rat feeding that Group II sorghums produced grains equal to those of the Group I sorghums. Sorghums not meeting the standards for Groups I, II, and III were not included in this feeding trial. Purple testa sorghums are a variable group.

Correlation coefficients and probabilities of PP, MV, PPO, TDMD, and IVDMD are given in Table 3. A strong correlation was found between PP and MV. The hybrid TX 2761 X TAM 2566 had a higher MV than expected. Negative but good correlations were found for PP and MV with PPO. The enzyme PPO plays a role in determining the molecular weight of the polyphenolic compounds. Weak correlations were found for PP, MV, and PPO with TDMD. Acceptable correlations were found for PP, MV, and IVDMD. PP and MV gave negative correlations with IVDMD. The correlations of TDMD with IVDMD indicated that the two animal species represented by these methods acted differently in the digestion of these grains.

CONCLUSIONS

Variations exist in the digestibility of grain in both the brown and non-brown sorghums. In general, the purple testa sorghums are as efficient as those without testae; those with brown testae are inferior. There is a higher

TABLE 3—Correlation Coefficients and Probabilities of PP, MV, PPO, TDMD, and IVDMD, 1980.

	MV	PPO	TDMD	IVDMD
PP				
r	0.968	−0.806	−0.376	−0.659
P	0.0001	0.0001	0.1020	0.0016
MV				
r	—	−0.861	−0.352	−0.623
P	—	0.0001	0.1276	0.0033
PPO				
r	—	—	0.174	0.617
P	—	—	0.4644	0.0038
TDMD				
r	—	—	—	0.223
P	—	—	—	0.2364

digestion efficiency in both nonbrown and brown sorghums with poultry than with ruminants.

These studies indicate that characteristics other than the amount of tannin affect digestion. The composition of tannin and the influence of other chemical factors in certain grains may play as great a role in digestibility as the amount. This study provided further evidence that the enzyme PPO plays a bigger role in sorghum grain than has been realized. The amount and composition of tannin appears to be related to PPO activity.

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