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Effect of Tannin on *In Vitro* Dry Matter and Protein Disappearance in Sorghum Grain[1]

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ABSTRACT

The purpose of this investigation was to determine the effect of tannin and added urea on *in vitro* dry matter disappearance (IVDMD) and *in vitro* protein disappearance (IVPD) in sorghum (*Sorghum bicolor* (L.) Moench) grain. IVDMD of low and high tannin genotypes after 48 hours of fermentation was 72.5 and 46.4% respectively. The addition of urea increased IVDMD to 93.4 and 76.2% for the low and high tannin genotypes. IVPD of low and high tannin genotypes was not affected by urea and averaged 88.2 and 69.6%, respectively. After 96 hours of fermentation the IVDMD of low and high tannin genotypes was 93.8 and 85.8%, respectively, indicating a true difference in digestibiltiy of about 8 percentage units.

The major factor limiting the utilization of high tannin sorghum is apparently the availability of protein. Undigestible protein and tannin complex may account for more than half of the difference in IVDMD between low and high tannin genotypes.

Additional index words: Urea, Protein digestibility, Nutritive value.

TANNINS in sorghum (*Sorghum bicolor* (L.) Moench) grain are responsible for several agronomically desirable traits, such as bird resistance, inhibition of preharvest seed germination, and weathering resistance (Harris, 1969). However, tannins have been shown to adversely affect the utilization of sorghum grain by monogastric and ruminant animals.

Chang and Fuller (1964) fed chicks diets of 50% sorghum and found that varieties with high levels (2.0%) of tannin resulted in growth retardation similar in magnitude to that caused by feeding equivalent levels of tannin acid. Other researchers have observed similar results when tannic acid was added to the ration of rats and chicks (Ringrose and Morgan, 1940, Handler and Baker, 1944; Glick and Joslyn, 1970).

Rostango (1972) found that amino acid availability of sorghum hybrid RS610 (low tannin) was decreased when tannic acid was added to growing chick rations. He also reported that high tannin sorghums had approximately 66% lower availability of amino acids than low tannin sorghums. Weight gain and feed efficiency of chicks on both high and low tannin sorghum diets significantly improved when essential amino acids were supplemented to the levels found in corn (*Zea mays* L.)

Nawar et al. (1970) showed significant variations in digestibility of dry matter and protein among varieties of sorghum grain fed to rats. *In vivo* dry matter digestibility ranged from 48.9 to 76.8%. McGinty (1968) reported that high tannin sorghum varieties had lower dry matter and protein digestibility than low tannin varieties when fed to ruminant animals. *In vivo* dry matter digestibility ranged from 50 to 71%. *In vitro* dry matter disappearance and *in vivo* dry matter digestibility were highly correlated. Stallcup and Davis (1962) conducted similar studies and found both *in vivo* dry matter digestibility and protein digestibility were lower with high tannin than with low tannin sorghums.

The purpose of this research was to determine 1) the effect of tannin in sorghum grain on *in vitro* dry

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matter and protein digestibility, and 2) the effect of supplemental nitrogen in the form of urea on the relationship between tannin content and dry matter and protein digestibility. Urea is a useful compound for this purpose because it effectively adds nitrogen in a rumen fermentation system without apparently altering the chemical composition of the materials being examined.

MATERIALS AND METHODS

Laboratory Procedure. Sorghum grain was harvested at a moisture content of 16 to 18%, dried to 10 to 12% moisture in a forced-draft drier, and ground in a Waring blender. The samples were stored at room temperature for subsequent laboratory analyses. Catechin equivalents, a measure of tannin concentration, were determined according to the vanillin-hydrochloric acid procedure of Burns (1963, 1971). Concentrations were expressed as catechin equivalents per 100 g dry grain.

In vitro dry matter disappearance (IVDMD) was determined using the Tilley and Terry (1963) two-stage technique for digestion of forage crops with modifications described by Barnes et al. (1971).

In vitro protein disappearance (IVPD) was determined by calculating the difference between the amount of total nitrogen (A.O.A.C., 1960) in the sample before and after *in vitro* fermentation. Protein was assumed to be equal to percent nitrogen × 6.25.

Experiment 1. Grain samples used to study the influence of tannin and urea on *in vitro* dry matter disappearance consisted of 24 commercial lines plus 10 India Sorghum (IS) lines selected from the World Collection of Sorghum (IS0062, IS0075, IS0184, IS0418, IS0616, IS2288, IS5437, IS7125, IS8165, and IS8361). Redland B and the 10 hybrids between Redlan A and the IS lines were also included. Duplicate laboratory determinations were made for tannin and *in vitro* dry matter disappearance. IVDMD was determined using 250-mg subsamples without the addition of urea and also using subsamples with the addition of 10 and 20 mg of urea per *in vitro* tube (50 ml). Dry matter disappearance was calculated after a 48-hour fermentation plus 24-hour pepsin digestion. Linear regression techniques were used to establish the effect of urea on the relationship between tannin and IVDMD. Separate regressions were calculated for the IS lines and their hybrids and for the 24 commercial entries. There was no significant difference between 10 and 20 mg of urea per tube; therefore, the data presented are averages of the two levels of urea.

Experiment 2. A second experiment was conducted using only four of the IS lines and their Redlan hybrids (IS0062, IS0418, IS8165, and IS0616). Two of the lines and their hybrids were low in tannin and two lines and their hybrids were high in tannin. IVDMD was determined on 250-mg subsamples following a 48-hour fermentation with rumen fluid and a 24-hour pepsin digestion. IVDMD was also determined, on another set of subsamples, following a 96-hour fermentation plus 24-hour pepsin digestion. During the 96-hour fermentation the samples were centrifuged, decanted, and reincubated with rumen fluid after 48 hours. *In vitro* protein disappearance was determined following a 48-hour fermentation with rumen fluid but without pepsin digestion. Undigested protein residues were adjusted by subtracting an amount of protein residue in blank rumen fluid fermentation. IVDMD and IVPD were determined with 0 and 15 mg of urea per tube. Duplicate IVDMD determinations were made on all samples in each laboratory run and two laboratory runs were made using rumen fluid collected on different days. IVPD was determined in only one laboratory run with duplicate determinations on each sample.

The data were analyzed statistically using analysis of variance techniques. The eight genotypes were considered a fixed variable, the two levels of urea (0 and 15 mg) as a fixed variable, and the variation within treatment combinations was considered an estimate of experimental error. The genotype source of variation was partitioned into parents (four IS lines) with three degrees of freedom, parents versus F_1's with one degree of freedom, and F_1's with three degrees of freedom. Significant differences between means were determined using the Newman-Keuls' test (Steel and Torrie, 1960).

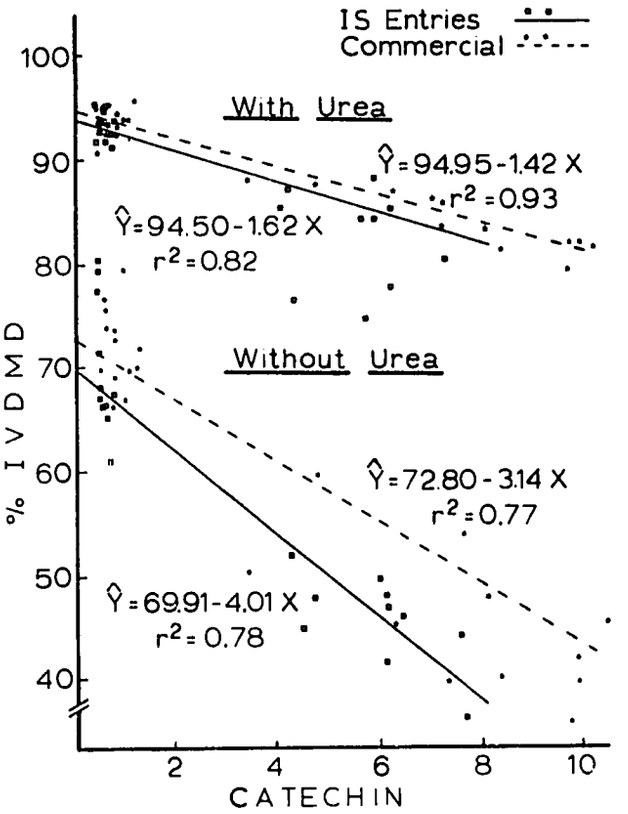

Fig. 1. Effect of urea on the relationship between catechin equivalents, measured as a percentage of dry weight, and *in vitro* dry matter disappearance in sorghum grain.

RESULTS AND DISCUSSION

Experiment 1. The addition of urea to the commercial entries reduced the regression coefficient for the regression of IVDMD on catechin equivalents from −3.14 to −1.42 ($P < .05$). Urea more than doubled the percent IVDMD of the highest catechin entries. Similar results were obtained with the IS lines and hybrids (Fig. 1). With these genotypes the addition of urea reduced the regression coefficient for the regression of IVDMD on catechin equivalents from −4.01 to −1.62. The differences in the regression coefficients with and without urea suggests that tannin, measured as catechin equivalents, is not inhibiting digestion of the sorghum grain directly but rather indirectly by reducing the amount of nitrogen available for bacterial growth and rapid substrate digestion. If tannin inhibited bacterial growth directly, then little or no increase in IVDMD would be expected by adding a small amount of urea.

The increase in maximum IVDMD (y-intercepts) with urea indicates that there is an insufficient amount of available nitrogen present even in the low tannin grain for maximum fermentation and dry matter disappearance. Schmid, Marten, and Roth (1969) observed similar effects on *in vitro* fermentation when urea was added to corn grain.

Experiment 2. Catechin equivalents, IVPD, and IVDMD (48-hour fermentation) for the low and high tannin genotypes are shown in Table 1. In all cases high tannin (catechin equivalents) was associated with

Table 1. *In vitro* dry matter disappearance and *in vitro* protein disappearance of eight sorghum genotypes with and without added urea after 48 hours of fermentation.

Genotypes	Catechin equivalents	IVDMD		IVPD	
		No urea	Urea	No urea	Urea
	%				
IS0062	0.29	80.7a*	95.4a	90.5a	93.0a
IS0418	0.27	68.6b	92.8a	85.8ab	87.3b
IS8165	4.28	55.4c	85.6b	76.1c	74.1c
IS0616	5.93	46.0d	77.7c	68.2d	65.2d
$\bar{X}$ lines	2.69	62.7	87.9	80.2	79.9
Redlan × IS0062	0.30	71.5b	92.9a	84.2b	91.5ab
Redlan × IS0418	0.22	69.3b	92.3a	86.8ab	86.2b
Redlan × IS8165	6.34	43.0d	81.2bc	61.4e	68.0d
Redlan × IS0616	7.46	41.3d	72.5d	58.1e	69.4cd
$\bar{X}$ Hybrids	3.58	56.3	84.7	72.6	78.8
$\bar{X}$ Low tannin genotypes	.27	72.5	93.4	86.8	89.5
$\bar{X}$ High tannin genotypes	6.00	46.4	79.2	70.0	69.2
LSD .05 for comparing means		2.20		1.86	

* Means in a column followed by the same letter do not differ significantly at the .05 level of probability using Newman-Keuls's test.

Table 2. Percent crude protein, 96-hour *in vitro* dry matter disappearance and undigestible protein in eight sorghum genotypes.

Genotypes	Catechin equivalent	Crude protein	96-hour IVDMD	Undigestible protein residue
	%	% of dry matter		
IS0062	0.29	13.78	94.8a*	1.13
IS0418	0.27	12.11	93.1a	1.62
IS8165	4.28	11.26	88.8b	2.80
IS0616	5.93	14.20	83.0c	4.73
$\bar{X}$ Lines	2.69	12.83	89.7	2.57
Redlan × IS0062	0.30	13.12	93.5a	1.60
Redlan × IS0418	0.22	13.12	93.9a	1.76
Redlan × IS8165	6.34	10.30	86.3c	3.64
Redlan × IS1616	7.46	13.81	85.0c	5.00
$\bar{X}$ Hybrids	3.58	12.58	89.7	3.00
$\bar{X}$ Low tannin entries	.27	13.03	93.8	1.53
$\bar{X}$ High tannin entries	6.00	12.24	85.8	4.04
LSD .05 for comparing means			.68	

* Means in a column followed by the same letter do not differ significantly at the .05 level of probability using Newman-Keuls's test.

Table 3. Sources of variation and associated level of significance in analysis of variance for *in vitro* protein disappearance and *in vitro* dry matter disappearance at 48 and 96 hours.

Source of variation	df	Level of significance: 48-hour IVDMD	IVPD	96-hour IVDMD
Genotypes	7	.01	.01	.01
Parent Lines	3	.01	.01	.01
Parent means vs F_1 means	1	.01	.01	n.s.
F_1's	3	.01	.01	n.s.
Level of urea	1	.01	.01	n.s.
Genotype × urea	7	.01	.01	n.s.
Error		24.50 46 df*	6.14 46 df	1.92 46 df*

* Runs × genotypes pooled with duplicates within runs

low IVDMD and IVPD. The difference between lines and F_1 hybrids may be partially due to the higher catechin equivalent content for the two high tannin hybrids than for the two high tannin lines. However, the difference in IVDMD and IVPD between IS0062 and Redlan × IS0062, two low tannin entries, cannot be attributed to differences in tannin only. This difference may be due to polyphenolyic compounds other than those detected by the vanillin-HCl method, possibly other pigments in the pericarp. For example, IS0062 has a colorless pericarp whereas its hybrid has a red pigmented pericarp inherited from the Redlan parent, also a low tannin line. The other low tannin line, IS0418, did not differ from its hybrid in IVDMD or IVPD. Plant breeders may be able to use genetic differences of this nature to improve the nutritional value of sorghum.

The addition of urea increased the IVDMD of low tannin genotypes from 72.5 to 93.4% ($P < .05$). IVPD was increased from 86.8 to 89.5%, a relatively small but statistically significant increase. Urea increased the IVDMD of high tannin genotypes from 46.4 to 79.2% ($P < .05$). Protein disappearance in the low tannin genotypes was generally not affected by the addition of urea. However, in the two highest tannin genotypes protein disappearance was apparently increased by the addition of urea ($P < .05$).

The lower protein disappearance of the high tannin genotypes as well as the relatively greater IVDMD increase in these genotypes with urea indicates that the amount of digestible protein may be a major factor limiting the utilization of high tannin sorghums by ruminant and monogastric animals. A recent report by Schaffert, Oswalt, and Axtell (1973) suggests that much of the undesirable effects of high tannin in sorghum on rat gains can be overcome with adequate protein supplementation.

The IVDMD of the eight genotypes after 96 hours of fermentation is shown in Table 2. Significant differences occurred between genotypes; however, the addition of urea had no significant effect (Table 3). Since urea had no effect on 96-hour IVDMD, the values in Table 2 are averages of both urea levels.

Urea may have had no effect on 96-hour IVDMD for several reasons. Enough endogeneous nitrogen may have been added with the second addition of rumen fluid to overcome differences due to amount of nitrogen available, the tannins may have been removed in the supernatant, or the first fermentation may have altered the tannin effects (Cummins, 1971). It is also possible that low nitrogen availability in the high tannin genotypes simply slowed the rate of disappearance and that 96 hours was sufficient time for even these genotypes to be completely digested. In any case, the lack of a significant increase in 96-hour IVDMD with urea indicates that available nitrogen was not a factor limiting the digestion of either the high or low tannin genotypes as was the case with 48-hour IVDMD.

If the 96-hour IVDMD can be assumed to represent the total amount of dry matter able to undergo digestion, then it appears that the true difference in IVDMD, and presumably *in vivo* digestibility, between the low and high tannin genotypes is about 8 percentage units. Difference in undigested protein residue (Table 2) accounts for 2.5 percentage units of this difference. Tannin is also presumably undigestible and while the average catechin equivalent content of the high tannin genotypes was 6.0%, the true tannin content is probably much less than 6.0%. Studies by Chang and Fuller (1964) have indicated that tannin in sorghum grain may range from 2.0 to 2.5% of the dry matter. If the true tannin content of the sorghum grain used in these studies was of this magnitude and if it is assumed to be undigestible, then as much as 62% of the difference in IVDMD between the low and high tannin genotypes can be attributed to an undigestible tannin-protein complex. The remainder of the difference in IVDMD between low and high tannin genotypes is presumably due to the presence of other undigestible compounds in the high tannin genotypes.

These studies suggest that the major factor responsible for the low *in vitro,* and presumably *in vivo* digestibility, in high tannin sorghum grain is the low digestibility or availability of protein in these genotypes. Perhaps much of the effect of tannin could be overcome with adequate protein supplementation.

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