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A Thesis

Submitted to the Faculty

of

Purdue University

by

Donn Paul Cummings

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

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To Tanya

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ABSTRACT

Cummings, Donn Paul. M.S., Purdue University, December 1973. Effect of Tannin Content and Testa of Sorghum bicolor (L.) Moench Grain on Nutritional Quality. Major Professor: J. D. Axtell.

The vanillin-hydrochloric acid (VHCl) and modified vanillin-hydrochloric acid (MVHCl) tests were used to determine the tannin content of 175 samples of grain sorghum harvested from individual plants in a segregating F_2 population from a cross between a high tannin and a low tannin line. Three groups of bulked samples were compiled on the basis of tannin content and presence or absence of testa. Weanling rats were fed the three sorghum diets for 14 days. The MVHCl test resulted in higher CE values than the VHCl test. Different or additional related polyphenolic compounds are probably being measured. Rat performance indicated that sorghum selections having testa present can perform as well as selections without a testa.

A second experiment was conducted to determine what level of tannin causes growth depression in weanling rats. NaOH-dehulled, high tannin sorghum was mixed in varying proportions with its whole counterpart to prepare a series of iso-nitrogenous diets differing only in tannin content. Lysine was supplemented to approximately 75 percent of the rat requirement. A significant decrease in weight gain, feed efficiency and protein efficiency with increasing levels of tannin was found.

The data indicated no threshold for tannin since any amount of tannin in the diet resulted in decreased rat weight gain.

INTRODUCTION

Increased protein quantity and quality of all major cereal grain crops is recognized as a necessary goal by most plant breeders and agronomists around the world to meet the ever-increasing demands of growing populations. Progress in protein improvement of grain sorghums has been hampered by a unique factor which decreases the nutritional value of some genotypes. A group of phenolic compounds known as tannins has been shown to reduce the biological value of some sorghums.

Several favorable agronomic traits of grain sorghum such as bird and weathering resistance have also been attributed to tannins. In many parts of the world it is a choice between a good yield from a less desirable high tannin sorghum or little or no yield from a more desirable low tannin sorghum because of bird damage. Therefore, many varieties presently available have been selected for higher tannin content to increase bird resistance. More information needs to be made available to the sorghum breeder concerning the effects of tannin so that selection criteria can be more accurately established for maximum benefit to mankind.

It is the purpose of this paper to 1) determine if the testa may be present without effecting the nutritional quality of grain sorghum and 2) establish what level of tannin in sorghum will cause decreased rat performance using iso-nitrogenous, isogenic grain sorghum diets.

LITERATURE REVIEW

Identification of Tannin in Grain Sorghum

Tannin compounds were identified in the pericarp and integument (testa) of Sudan grass and Johnson grass caryopses by Harrington and Crocker in 1923. Wall and Ross (1970) have noted that sorghum varieties with pigmented testa and dark red- or brown-colored pericarp are generally higher in phenolic compounds (tannins) than lighter-colored varieties with white, yellow, or red pericarps. Harris (1969) tested 20 sorghum hybrids classified by seed color and found that the brown-seeded hybrids were approximately three percent higher in tannin than the red- or yellow-seeded hybrids. Strumeyer and Malin (1969) identified a general protein denaturant present in Leoti sorghum as a series of oligomeric condensed tannins of the leucocyanidin group varying in degree of polymerization. The principal tannin in grain sorghum has been identified as a leuco-anthocyanin which upon heating with mineral acid yields leuteoforol (leucoluteolinidin) 3', 4, 4', 5, 7 -pentahydroxyflavan (Bate-Smith and Rasper, 1969).

Bate-Smith and Rasper (1969) have identified three distinct layers of the sorghum caryopsis which may contain tannin and other pigments: the epicarp, the hypocarp (outer layers of the pericarp) and the testa (an inner layer separated from the pericarp by a colorless mesocarp). Any one of these layers may be completely absent and their presence or

absence is genetically determined (Quinby and Martin, 1954). Maxson, et al. (1972) have reported that the genes, B_1B_2S, which control pericarp color and presence or absence of a pigmented testa significantly affect tannin content. They observed that sorghums with a pigmented testa had high tannin contents associated with them as determined by two different methods of tannin analysis. Wanjari and York (1972) have also reported on pigment inheritance in sorghum grain confirming the results of earlier workers.

It is now possible for plant breeders to manipulate the tannin content of grain sorghum with relative ease, but it remains unclear as to what level of tannin is desirable, since factors having both beneficial and deleterious aspects have been associated with tannin.

Factors Associated with Tannin in Grain Sorghum

Many researchers have presented data supporting "bird resistant" qualities associated with the brown (high tannin) sorghums (York and Thurman, 1962; Harris et al., 1964; Voigt, 1966; Harris, 1969; Niehaus and Schmidt, 1970; Tipton et al., 1970; and McMillian et al., 1972). In a recent study by McMillian et al. (1972) in which 142 sorghum lines were tested for bird resistance, a negative correlation ($r = -0.622$) between bird damage and tannin content of the seed was found. In addition, a highly significant positive correlation ($r = 0.520$) between seed color and tannin was shown, indicating that dark brown color was associated with high tannin. Harris (1969) has stated that the bird resistance during the milk and dough stages of maturity is a result of the astringent flavor of anthocyanogens (Blessin et al., 1963) which

have been suggested as precursors of condensed tannins (Roux, 1957; Roux and Evelyn, 1958).

Weathering, deterioration of seed quality due to weather conditions including preharvest seed germination, is reportedly less serious in high tannin sorghums (Harris, 1969; Harris and Burns, 1970; York and Thurman, 1962). The role of tannin in weathering resistance is not well understood, although Harris and Burns (1970) have suggested possible ways in which tannins may function to retard preharvest seed germination. Seed molding has also been observed to be less severe in high tannin sorghums (Harris et al., 1964; Harris, 1969).

Inhibition of many different enzymes has been reported and may be due to the protein binding nature of tannins. An amylase inhibitor from Loeti sorghum was characterized by Miller and Kneen (1947) and later identified as a series of condensed tannins by Strumeyer and Malin (1969). Smart et al. (1961) found that Sericea tannins inhibited cellulase activity. Goldstein and Swain (1965) reported the inhibition of B-glucosidase and other enzymes by condensed tannins from wattle bark. Tamir and Alumont (1969) demonstrated that tannins extracted from carobs inhibited digestive enzymes including trypsin, α-amylase and lipase. They suggested that the inhibition is a result of the tannins binding with the enzyme precipitating it out of solution.

Rostango et al. (1973a) noted an increase in leg abnormalities in chicks maintained on a high tannin sorghum diet. Whether or not these observations are related to tannin content or other factors has not been clearly established.

Morton (1970) has implicated consumption of tannins in grain sorghum and other plant tannins as a possible factor relating to the incidence of esophageal cancer in many areas of the world. Tannins can cause gastrointestinal inflammation and may be tumorigenic.

Nutritional Aspects of Tannin and Tannic Acid

Researchers have shown that tannin or tannic acid in animal diets often results in reduced weight gain. Ringrose and Morgan (1940) and Rayudu et al. (1970) reported that tannic acid fed as two percent of the diet resulted in reduced growth of chicks. Tannic acid fed to rats as five percent of the diet gave weight gains of approximately 50 percent of the control (Joslyn and Glick, 1969). Lease and Mitchell (1940) found that rats could tolerate up to five percent tannic acid mixed in a good ration, but that higher levels caused marked growth depression and lower hemoglobin. Handler and Baker (1944) observed reduction in rat growth when levels of one to two percent tannic acid were administered.

Lower weight gains and reduced feed efficiency for chicks fed high tannin sorghum diets is well documented (Chang and Fuller, 1964; Connor et al., 1969; Rostango et al., 1973a). Chang and Fuller (1964) found that tannic acid added to a low tannin diet up to the level of the high tannin sorghum resulted in equivalent growth depression; however, Rostango et al. (1973a) in a similar experiment found that equivalent levels of tannic acid did not result in the same magnitude of growth depression as high tannin sorghum. Fuller et al. (1966) concluded that dietary levels of tannin between 0.64 and 0.84 percent

(from grain sorghum) are required to show a depressing effect on growth rate in chickens.

Oswalt (1973) compared high and low tannin groups of grain sorghum genotypes at near iso-nitrogenous levels and found that rat weight gain was generally higher in the low tannin groups at a given level of crude protein and lysine content. Jambunathan and Mertz (1973) fed rats diets prepared to contain equal amounts of protein, oil, minerals and fiber, but differing in tannin content. The high tannin diet supported lower weight gain and resulted in reduced protein efficiency ratios when compared to the low tannin diet.

Many workers have reported complete or partial elimination of the toxic effects of tannin and tannic acid through dietary supplementation with various compounds. Chang and Fuller (1964) found that choline and methionine supplementation in chick diets completely eliminated the growth depression produced by tannin in grain sorghum but only partially eliminated the toxicity of tannic acid. Vohra et al. (1966) were not able to reproduce these results and reported that methionine, choline, betaine, or ornithine did not reduce the growth depressing effects of tannic acid. Connor et al. (1969) were able to confirm the work of Chang and Fuller (1964) in part. They found that methionine and choline partially alleviated the growth depressing effects of tannic acid, but did not completely eliminate the growth depressing effects of tannin in grain sorghum. A mixture of arginine, ornithine and methyl donors like methionine and choline partially alleviated the growth depression induced by tannic acid in work by Rayudu et al. (1970). Additional protein and iron were shown to reduce the toxicity of tannic acid in the

rat by Lease and Mitchell (1940). McGinty (1968a) has reported that the use of polyethylene glycol alleviated the decrease in digestibility caused by tannin in sorghum. Armstrong et al. (1973) have shown that polyvinyl pyrrolidone increases chick performance when added to rations containing high tannin sorghums or tannic acid supplementation.

Tannin and tannic acid have been shown to reduce the digestibility of sorghum and other plant materials by many researchers using several different techniques. McGinty (1968b) tested eight sorghum varieties using two *in vitro* techniques and an *in vivo* method with steers and found that the two varieties having a testa present were significantly lower in digestibility. He further noted that addition of soluble materials from the pericarps of these two lines to rumen fluid caused a significant increase in the amount of nitrogen precipitated (McGinty, 1968a). Vohra et al. (1966) indicated that dietary tannic acid resulted in reduced nitrogen retention in chicks. Increased quantities of insoluble nitrogen in the digestive system of rats fed tannins extracted from carob pods has been reported by Tamir and Alumont (1970). They concluded that the increased insoluble nitrogen was due to nonspecific binding of proteins to tannins. Glick and Joslyn (1970) concluded that increased fecal nitrogen of rats fed tannins results from endogenous rather than dietary proteins. Schaffert (1972) reported that reduced IVDMD of high tannin sorghum grain when compared to low tannin sorghum grain was partially due to increased nitrogen utilization associated with a non-digestible protein-tannin complex. Jambunathan and Mertz (1973) have shown that high and low tannin sorghums exhibit different protein fractionation patterns consistent with the idea of protein-tannin

complex formation. Although a number of researchers have related tannin to lower protein digestibility in sorghum, little is known about the actual mechanisms which are involved.

It is generally accepted that tannin in grain sorghum reduces the nutritional quality in at least three ways: 1) lower digestibility due to reduced protein availability, 2) toxic effects, and 3) reduced feed consumption (although some controversy exists in the literature).

The Role of Lysine in Evaluating Tannin in Grain Sorghum in Rats

Axtell et al. (1973) have reported that the mean value for lysine for 522 sorghum lines from the world collection is 2.14 percent (as percent of protein) and protein averages 12.6 percent making an all sorghum diet approximately 0.27 percent lysine. The lysine requirement of the rat is one percent of the sample (Mertz, 1969). Because of this deficiency, lysine is considered the first limiting amino acid in sorghum. Axtell et al. (1973) fed high and low tannin sorghum lines to weanling rats with and without supplementation with lysine-HCl. They observed significant increases in 14-day weight gain for the low tannin lines, but very little response to lysine supplementation was observed in the high tannin line indicating that other factors are first limiting in high tannin sorghums. In another experiment by these workers, lysine content of 17 low tannin lines was shown to be related to 14-day rat weight gain in unsupplemented feeding trials while no relationship was found between lysine content and rat performance in the rations prepared from high tannin lines.

METHODS AND MATERIALS

Experiment I

The purpose of this experiment was to determine if the presence or absence of the testa is a useful indicator of biological value of grain sorghum. In addition, the relationships between the VHCl and MVHCl tests and sorghum quality were investigated.

A segregating F_2 population selected from a cross between a low tannin sorghum, Purdue 954 062 (0.15 cat. eq.), and a high tannin line, IS 2942 (5.53 cat. eq.), were planted at the Purdue Agronomy Farm in May, 1972. At maturity the F_2 seed was harvested from individual plants and dried to approximately 12 percent moisture. The grain from 175 plants was classified for presence or absence of testa by scraping the outer layers of several seeds from each sample and looking for the dark brown testa (or subcoat). Microscopic examination of thin sections of several grains verified that this is an accurate method of screening for the presence of a pigmented testa.

Tannin Analysis

Each sample was analyzed for tannin by two methods, the vanillin-hydrochloric acid method (VHCl) (Burns, 1963, 1971) and the modified vanillin-hydrochloric acid method (MVHCl) (Maxson and Rooney, 1972). The VHCl test was changed to include two hours on a shaker before setting overnight during the extraction procedure rather than "swirling

occasionally" during a 20 to 28-hour extraction period as described by Burns (1971). This resulted in slightly higher values (recorded as catechin equivalents) indicating a more complete extraction.

Rat Diets

On the basis of the two tannin analyses and the testa data three groups of composite grain samples were prepared by bulking individual heads. Group I consisted of those samples lacking a brown subcoat with values less than 1.0 catechin equivalent (CE) on both the VHCl and MVHCl tests. Group II consisted of those samples having a testa present and with VHCl values less than 1.0 CE and MVHCl values greater than 2.0 CE. Group III contained all samples with a testa and values of greater than 2.0 CE on both the VHCl and MVHCl tests. Some samples were not within these limits and were not included in any of the groups. The grain was ground, thoroughly mixed, and analyzed for protein as percent of dry sample (P), lysine as percent of protein (L) and lysine as percent of sample (PxL). Three diets were prepared using the three groups of ground sorghum grain as the only source of protein. Each 100g of the diet contained 94g sorghum, 4g of mineral mix[1], and 2g of vitamin mix[2].

Rat Feeding[3]

Thirty-six male weanling rats ranged in initial weight from 44 to 63g and averaging 52g were used in two replications of a 14-day feeding

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[1] Hawk-Oser salt mixture number three, Nutritional Biochemicals, Inc., Cleveland, Ohio.
[2] Vitamin supplement, General Biochemicals, Chagrin Falls, Ohio.
[3] Rat feeding trials were conducted by Shuman Chemical Laboratory. Battle Ground, Indiana.

trial. The replications were started one week apart. Six rats were assigned to each diet within one replication so that the average weights for each of the three treatment groups were approximately equal. The rats were placed in individual cages and feed and water were made available ad libitum. At the end of the trial, weight gain was determined for each rat individually and feed consumption was determined for each group of six rats collectively. In the final analysis the average weight gain per six rats was used. Feed efficiency ratios (FER, grams of feed consumed per gram of gain) and protein efficiency ratios (PER, grams of gain per gram of protein consumed) were calculated.

Statistical Analysis

Standard correlation analysis according to Steel and Torrie (1960) was used.

Experiment II

The objectives of this experiment were to determine if the compounds measured by the VHCl test are responsible for reduced nutritional quality of sorghum and to establish the concentration of tannin necessary to cause growth reduction in rats.

A large quantity of dark brown high tannin (5.24 CE) sorghum (IS 8260) grown in the Purdue/AID Puerto Rico winter nursery in 1972 was obtained. Some of the grain was dehulled using the sodium hydroxide method described by Blessin (1971) to remove the pericarp and testa layers which contain most of the tannin in the sorghum caryopsis. The procedure is included here for completeness:

Stir constantly through all procedures:

a) Temper samples in 140° F water for five minutes.
b) Heat in 20 percent NaOH at 160° F for five minutes.
c) Drain, wash six times with 140° F water.
d) Neutralize with 5 percent glacial acetic acid at 140° F during a five minute period.
e) Wash five times with 140° F water.
f) Drain and dry at 113° F to approximately 12 percent moisture.

Chemical Analyses

The whole and dehulled grain was ground and analyzed for tannin content using the VHCl test (Burns, 1963, 1971). In addition, samples were analyzed for protein as percent of sample by multiplying 6.25 times the percent nitrogen determined by the micro-Kjeldahl technique. The lysine content as percent of protein was determined by ion-exchange resin chromatography.

Rat Diets

Eight diets were prepared using the whole and dehulled grain as the sole source of protein. All diets contained one percent vitamin mix and four percent mineral mix and six of the diets were supplemented with 0.5 percent lysine as lysine-HCl. The whole and dehulled grain were mixed in varying proportions to yield a gradient of tannin levels ranging from 0.11 to 4.83 CE. The final composition of each diet is presented in Table 1.

Rat Feeding

Weanling male rats of the Wistar Inbred Colony, Biochemistry Department, Purdue University were used for this experiment. Ten rats weighing approximately 50g each were randomly assigned to each treatment

Table 1. Composition of rat diets used in Experiment II.

Components (g)	Diet (Contents per 100 g)							
	A	B	C	D	E	F	G	H
Whole Grain			33.03	47.19	56.62	66.06	94.37	95.00
Dehulled Grain	95.00	94.37	61.34	47.18	37.75	28.31		
Vitamin Mix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral Mix	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Lysine - HCl		0.63	0.63	0.63	0.63	0.63	0.63	
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

group and group weights were adjusted so that the mean initial weight in each treatment was approximately equal. The rats were individually caged in feeding shelves in a climate controlled room. Feed and water were made available ad libitum. All lysine supplemented diets were fed for 28 days while unsupplemented diets were fed for only 14 days due to limited grain supply. Feed consumption and weight gain were recorded for individual rats at two and four weeks. Feed efficiency ratios and protein efficiency ratios were calculated as in Experi---- I.

Statistical Analysis

Standard procedures for correlation analysis, analysis of variance, and Newman-Keuls test were used in interpreting the data (Steel and Torrie, 1960). In performing F-tests on mean squares from analysis of variance, interaction terms which were not significant were pooled with the residual to obtain a better estimate of the true error.

RESULTS AND DISCUSSION

Experiment I

Comparison of MVHCl and VHCl Tests for Tannin

Catechin equivalent (CE) values determined by the modified vanillin-hydrochloric acid (MVHCl) test (Maxson and Rooney, 1972) were generally higher than CE values for the same sample using the vanillin-hydrochloric acid (VHCl) test (Burns, 1971). Catechin equivalent values of 175 samples for both the VHCl and the MVHCl tests are given in Table 2. The mean VHCl value for samples without testa was 0.428 CE while the mean MVHCl value for the same group was 0.705 CE. Likewise, the mean values of the samples with testa were 1.03 CE for VHCl and 3.36 CE for MVHCl (Table 3).

The methanol extract was generally darker red in color when acidified with one percent HCl. Color change upon addition of the vanillin-HCl reagent was more marked in the non-acidified extracts.

The VHCl and MVHCl tests were not significantly correlated ($r = 0.248$) when only the samples lacking testa were considered, but were highly significantly ($p < 0.01$) correlated ($r = 0.661$) when only the samples with testa were considered. The overall correlation of 175 samples was $r = 0.655$ ($p < 0.01$).

It appears likely that the MVHCl test measures different or additional related phenolic compounds than is measured by the VHCl test.

Table 2. Tannin content (CE) of 175 samples of sorghum determined by the VHCl and MVHCl tests.

Sample #	Testa	Catechin Equivalents VHCl	Catechin Equivalents MVHCl	Group	Sample #	Testa	Catechin Equivalents VHCl	Catechin Equivalents MVHCl	Group
001	Absent	0.33	0.53	I	044	Present	0.60	3.44	II
002	Absent	0.39	0.48	I	045	Present	0.39	2.74	II
003	Present	3.63	4.33	III	046	Absent	0.42	1.20	*
004	Present	0.71	2.52	II	047	Present	0.76	2.51	II
005	Present	1.40	3.88	*	048	Present	0.50	1.93	*
006	Absent	0.67	1.05	I	049	Absent	0.21	0.49	I
007	Present	1.19	2.10	*	050	Present	0.65	2.18	II
008	Absent	0.44	0.62	I	051	Absent	0.18	0.50	I
009	Present	0.29	1.60	*	052	Present	1.85	3.42	III
010	Absent	0.15	0.48	I	053	Present	0.59	2.86	II
011	Present	0.15	3.02	II	054	Absent	0.26	0.56	I
012	Absent	0.12	0.24	I	055	Absent	0.40	0.59	I
013	Present	1.76	4.02	III	056	Present	0.32	2.60	II
014	Present	0.44	3.01	II	057	Absent	0.29	0.50	I
015	Present	0.19	1.48	*	059	Present	0.72	2.35	II
016	Absent	0.06	0.13	I	060	Absent	0.44	0.62	I
017	Absent	0.13	0.25	I	062	Present	0.51	3.01	II
018	Absent	0.43	0.59	I	063	Present	0.80	4.90	II
019	Absent	0.23	0.56	I	064	Present	0.33	2.32	II
020	Present	0.44	2.35	II	065	Present	3.28	4.84	III
021	Absent	0.22	0.56	I	066	Present	1.47	2.79	*
022	Present	0.43	2.44	II	067	Present	0.69	1.97	*
023	Present	2.32	3.75	III	068	Present	0.69	1.91	*
024	Present	0.98	2.94	II	069	Absent	0.66	0.60	I
025	Present	0.46	2.80	II	070	Present	0.46	2.25	II
026	Present	0.36	1.20	*	071	Absent	0.47	0.61	I
027	Absent	0.08	0.34	I	072	Present	0.33	2.35	II
028	Absent	0.34	0.69	I	073	Absent	0.15	0.48	I
029	Present	0.31	1.81	*	074	Present	2.67	4.98	III
030	Present	2.42	4.34	III	075	Present	1.10	4.17	*
031	Present	0.70	2.04	II	076	Absent	0.53	0.80	I
032	Present	0.40	1.62	*	077	Absent	0.70	0.82	I
033	Present	0.28	0.98	*	078	Absent	0.99	0.62	I
034	Present	1.78	3.79	III	079	Present	0.31	1.91	*
035	Present	0.56	2.18	II	080	Present	0.50	2.20	II
036	Present	1.20	2.94	*	081	Absent	0.32	0.61	I
037	Present	0.59	1.81	*	082	Present	1.00	3.32	*
038	Absent	0.82	1.32	*	083	Absent	0.14	0.62	I
039	Present	0.75	1.79	*	084	Present	1.06	2.71	*
040	Present	0.56	1.43	*	085	Present	0.16	4.40	II
041	Present	1.26	2.81	*	086	Absent	0.17	0.68	I
042	Absent	0.30	0.59	I	087	Absent	1.08	1.22	*
043	Absent	0.32	0.73	I	088	Present	2.32	5.45	III

Table 2, cont.

Sample #	Testa	Catechin Equivalents VHCl	Catechin Equivalents MVHCl	Group	Sample #	Testa	Catechin Equivalents VHCl	Catechin Equivalents MVHCl	Group
089	Present	0.69	2.25	II	134	Present	2.24	4.92	III
090	Absent	0.95	0.89	*	135	Absent	0.26	0.83	I
091	Present	3.00	5.35	III	136	Absent	0.30	0.94	I
092	Present	0.75	3.90	II	137	Present	0.98	3.96	II
093	Present	0.92	2.92	II	138	Absent	0.34	0.94	I
094	Present	1.04	2.53	*	139	Absent	0.22	0.88	I
095	Absent	0.31	0.60	I	140	Absent	0.24	0.72	I
096	Present	0.52	2.24	II	141	Present	0.38	3.39	II
097	Absent	0.21	0.73	I	142	Present	0.42	3.55	II
098	Present	0.34	1.98	*	143	Present	1.24	3.78	*
099	Absent	0.46	0.31	I	144	Present	0.59	3.49	II
100	Present	0.33	3.25	II	145	Absent	0.45	0.90	I
101	Present	0.39	6.68	II	146	Present	0.78	3.49	II
102	Present	0.71	3.84	II	147	Present	3.20	6.26	III
103	Present	0.75	3.19	II	148	Present	5.53	7.84	III
104	Absent	0.28	0.57	I	149	Absent	0.59	1.78	*
105	Present	2.08	2.26	III	150	Present	0.59	2.72	II
106	Present	0.95	2.29	II	152	Present	0.50	2.38	II
107	Present	0.47	4.69	II	153	Absent	0.22	0.85	I
108	Present	0.50	2.07	II	154	Present	0.96	3.62	II
109	Absent	1.12	0.52	*	155	Absent	0.39	1.21	*
110	Present	0.74	3.99	II	156	Present	0.58	3.23	II
111	Absent	0.20	1.33	*	157	Present	0.40	2.71	II
112	Present	1.35	2.20	*	158	Present	1.40	4.04	*
113	Absent	0.30	0.55	I	159	Present	0.95	3.57	II
114	Present	0.42	5.04	II	160	Present	0.57	2.71	II
115	Present	1.05	1.64	*	161	Present	1.18	5.56	*
116	Present	0.62	2.92	*	162	Present	1.69	5.84	III
117	Absent	0.22	0.27	I	163	Present	1.63	5.44	III
118	Present	0.38	2.62	II	164	Absent	0.34	0.91	I
119	Present	0.93	2.94	II	165	Present	0.51	3.23	II
120	Present	0.44	3.96	II	166	Present	0.54	3.62	II
121	Present	0.94	3.99	II	167	Present	0.31	3.60	II
122	Present	0.46	3.90	II	168	Present	0.35	3.10	II
123	Present	0.74	4.30	II	169	Absent	0.14	0.64	I
124	Present	0.45	4.40	II	170	Present	0.46	3.13	II
125	Present	0.45	2.56	II	171	Present	0.46	3.62	II
126	Absent	1.37	0.57	*	172	Present	0.35	2.84	II
127	Present	2.14	5.77	III	173	Absent	0.42	0.85	I
128	Present	2.55	4.99	III	174	Present	2.16	5.07	III
129	Present	3.45	4.92	III	175	Present	2.30	6.05	III
130	Absent	2.47	0.78	*	176	Absent	0.30	0.91	I
131	Absent	0.30	0.73	I	177	Present	3.07	6.11	III
132	Absent	0.38	0.72	I	178	Present	2.15	5.51	III

Table 2, cont.

Sample #	Testa	Catechin Equivalents VHCl	MVHCl	Group	Sample #	Testa	Catechin Equivalents VHCL	MVHCl	Group
179	Absent	0.26	0.82	I					

* not included in rat feeding trials since these samples were not within the arbitrary limits for bulking.

The correlation of these t o tests for tannin with biological value will determine their usefulness to the sorghum breeder.

Bulking of Samples for Rat Feeding

The criteria for bulking samples were chosen arbitrarily based on the distribution of CE values to determine differences between these two tests relative to weanling rat performance. When the testa was absent, VHCl values were rarely greater than 1.0 CE and MVHCl values, although generally higher, were also seldom greater than 1.0 CE. When the testa was present, the VHCl values tended to fall into two categories, less than 1.0 CE and greater than 2.0 CE, with few samples falling between 1.0 and 2.0 CE. The MVHCl values were generally greater than 2.0 CE when the testa was present averaging 3.13 CE in the group with VHCl values less than 1.0 CE and increasing to 5.01 CE in the group with VHCl values greater than 2.0 CE. The outward appearance of the bulked samples showed no obvious visible differences with the exception that Group I had a slightly higher concentration of white grains. The

Table 3. Comparison of mean VHCl and MVHCl values for 175 samples of grain sorghum from a segregating F_2 population.

	N	VHCl		MVHCl		Correlation Coefficient
		Mean CE	sd	Mean CE	sd	
Testa Absent	60	0.428	0.382	0.705	0.294	0.245**
Testa Present	115	1.032	0.907	3.361	1.311	0.661**
Total	175	0.824	0.820	2.451	1.659	0.655

** Significantly different from zero at the 0.01 probability level.

analyses of the bulked sorghum samples used to make up the three diets in Experiment I are presented in Table 4.

Weanling Rat Response to the Three Diets

The mixed diets were analyzed for protein and lysine concentration, and catechin equivalent values were determined with the VHCl test. Small variations in protein (ranging from 11.3 to 11.7 percent) and lysine (ranging from 1.74 to 1.84 percent of protein) were found (see Table 5). These differences vary with CE level and their effect on rat performance is completely confounded with CE and assumed to be small. Table 5 gives 14-day feed consumption, weight gain, feed efficiency ratios (FER), and protein efficiency ratios (PER). The average feed consumption per rat was about the same for each group. Tannin (CE) did not effect feed consumption since rats fed Group III (3.20 CE) rations consumed nearly the same amount as those fed Group I (0.24 CE) diets. Figure 1 illustrates the 14-day rat weight gain for each treatment group. Lysine is the first limiting amino acid in all three diets and the low lysine levels were responsible for the overall low weight gain for all treatments. Group III rats gained less and were considerably less efficient in feed conversion and protein utilization than either Group I or Group II rats which was expected due to the higher tannin content of the diet. Group II is especially significant since it represents a diet composed of sorghum grains with testa which performed as well as sorghum grains without testa (Group I) in unsupplemented rat feeding trials. This is evident in FER and PER values also (Table 5). Differences in CE values between Group I and Group II diets measured by the MVHCl test were not reflected in the rat growth

Table 4. Analyses of bulked sorghum samples used in preparation of rat diets for Experiment I.

Group	Testa	Number of Samples	Average VHCl CE	Average MVHCl CE	Protein % of Sample	Lysine % of Protein	Lysine % of Sample
I	Absent	50	0.32	0.63	10.85	1.48	0.16
II	Present	63	0.57	3.13	10.63	1.49	0.16
III	Present	24	2.53	5.01	10.50	1.64	0.17

Table 5. Analyses of rat diets in Experiment I and 14-day rat trial data.

Group	Catechin Equiv-alents	Protein % of Sample	Lysine % of Protein	Lysine % of Sample	Feed Con-sumption	Weight Gain	Feed Efficiency Ratios	Protein Efficiency Ratios
I	0.24	11.7	1.84	0.22	83	5.62	14.89	0.58
II	0.52	11.4	1.83	0.21	87	6.25	16.27	0.63
III	3.20	11.3	1.74	0.20	84	2.43	34.92	0.26

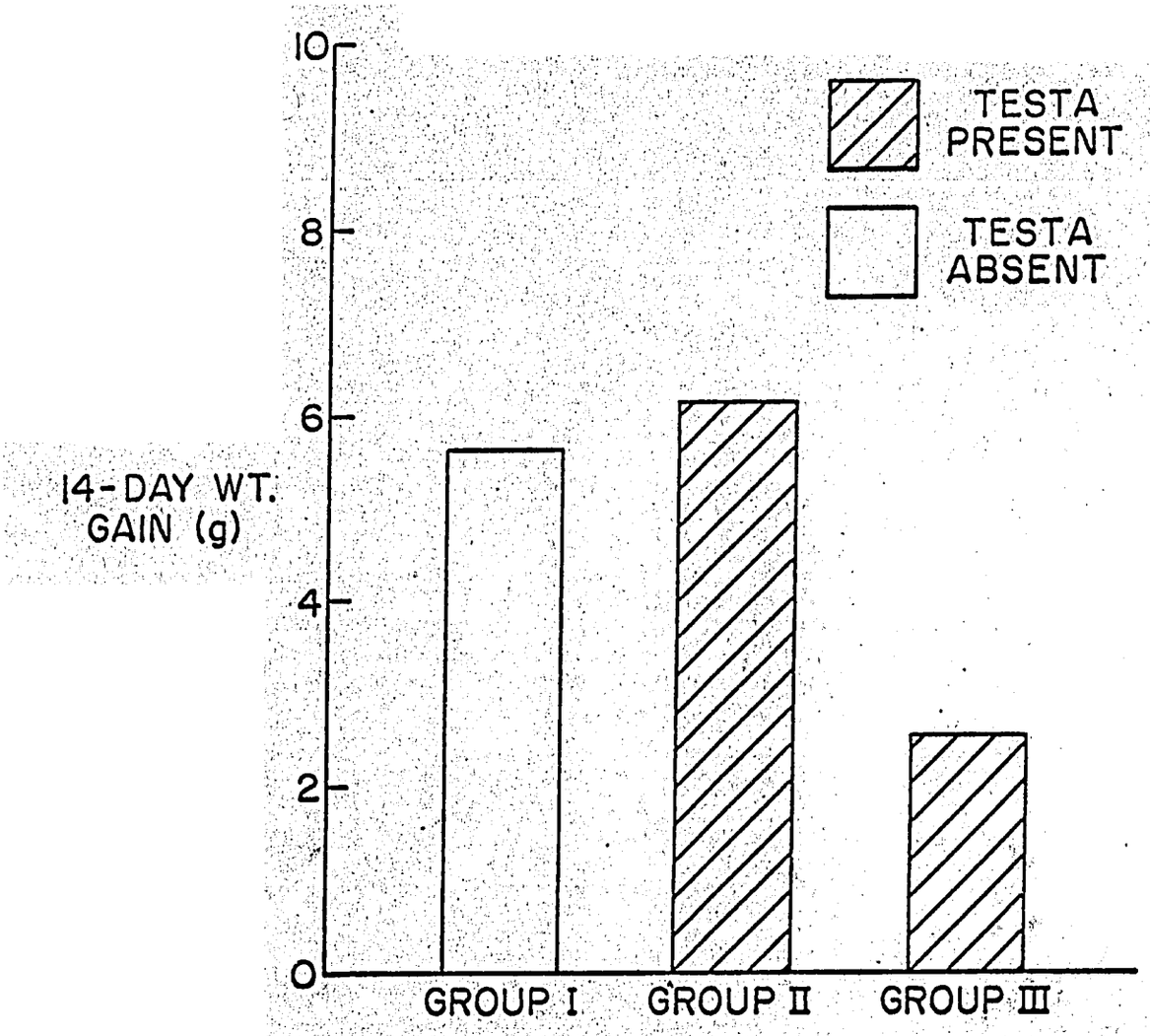

Figure 1. Comparison of 14-day weight gain on three diets in Experiment I.

data while the CE values for the VHCl test were consistent with these data. The MVHCl test was also more highly correlated with the presence or absence of the testa than the VHCl test. Since the presence or absence of the testa was shown not to be a good indicator of sorghum nutritional quality, it was concluded that the MVHCl test is less effective than the VHCl test for predicting the nutritional quality of grain sorghum.

The association that is often made by sorghum breeders between tannin (as measured by the VHCl method) and the testa is not absolute. Group II represents 36 percent of the plants sampled from a segregating E_2 population which have testa present but remain low in tannin. Whether or not these hybrids have retained some of the agronomic characteristics commonly associated with a brown subcoat such as bird and weathering resistance remains to be determined.

Experiment II

The Effect of Dehulling and Lysine Supplementation of High Tannin Grain Sorghum on Weanling Rat Growth

The sodium hydroxide dehulling process was effective in removing the phenolic compounds measured as catechin equivalents by the VHCl test in agreement with Blessin (1971). However, the percent crude protein increased in value approximately one percent (Table 6) in contrast to reports of no change in protein content by others (Blessin, 1971 and Oswalt, 1973). An increase in percent protein would be expected when the pericarp tissue is removed due to the low protein concentration of the pericarp. Lysine content of the grain did not change.

Table 6. Analyses of grain and diets used in Experiment II.

	IS 8260		Complete Diets							
	Whole	Dehulled	A	B	C	D	E	F	G	H
Catechin Equivalents (CE)	5.24	0.04	0.11	0.14	0.67	1.20	1.49	2.02	3.66	4.38
Protein % of Sample (P)	10.46	11.40	12.03	12.47	12.40	12.40	12.43	12.07	11.77	11.53
Lysine % of Protein (L)	1.70	1.78	2.01	5.95	5.69	5.60	5.84	5.90	6.39	2.12
Lysine % of Sample (PxL)	0.18	0.20	0.24	0.74	0.71	0.69	0.72	0.71	0.75	0.24

Dehulled IS 8260 grain rations when fed to weanling rats resulted in a significant increase in weight gain, feed efficiency, and protein efficiency when compared to the whole-grain diets. Feed consumption remained the same for the whole and dehulled grain when unsupplemented with lysine (Table 7). When lysine was added to the whole grain ration (up to 75 percent of rat requirement), no significant changes in rat performance were observed. When rats were fed the dehulled sorghum diet with added lysine large increases in feed consumption, weight gain, feed efficiency, and protein efficiency occurred above any differences due to dehulling (Table 7 and Figure 2). These findings are in agreement with similar findings of Axtell et al. (1972) and Oswalt (1973) indicating that lysine is the first limiting factor in grain sorghum diets except when tannin is present at high concentrations causing protein to become first limiting due to tannin-protein complex formation.

The analysis of variance in Table 8 indicates a highly significant effect and interaction for NaOH dehulling and lysine supplementation on feed consumption, weight gain and protein efficiency ratios. Individual rats contributed significantly to the total variation in feed efficiency ratios while lysine supplementation was not significant. The reason for this is not evident and protein efficiency ratios may be the variable of choice in determining biological value since they agree better with the weight gain data.

Table 7. Effect of dehulling and lysine supplementation of high CE grain sorghum on weanling rat growth in a 14-day feeding trial

	Feed Consumption (g)	Weight Gain (g)	Feed Efficiency Ratios	Protein Efficiency Ratios
Whole IS 8260	88.8	2.3	44.97	0.32
Whole IS 8260 Lysine Added	77.5	3.1	36.47	0.37
Dehulled IS 8260	85.6	12.2	7.45	1.17
Dehulled IS 8260 Lysine Added	120.4	40.7	2.99	2.70

Changes in Measurable Tannin Content (CE) Upon Addition of Vitamin and Mineral Mixes and Lysine-HCl

When one percent vitamins and four percent minerals were added to whole IS 8260 (diet H, Table 6), a decrease in CE (from 5.24 to 4.38 CE) was observed. This much change is greater than could be explained due to dilution and suggests that some of the tannin compounds may be combining with some component of these mixes causing a change in form rendering the tannin no longer detectable by the VHCl test. An additional decrease in CE was observed when 0.625 percent lysine-HCl was added (diet G, Table 6) indicating that tannin may also bind with lysine (Figure 3). Further investigation is needed to determine the mechanisms responsible for these observations.

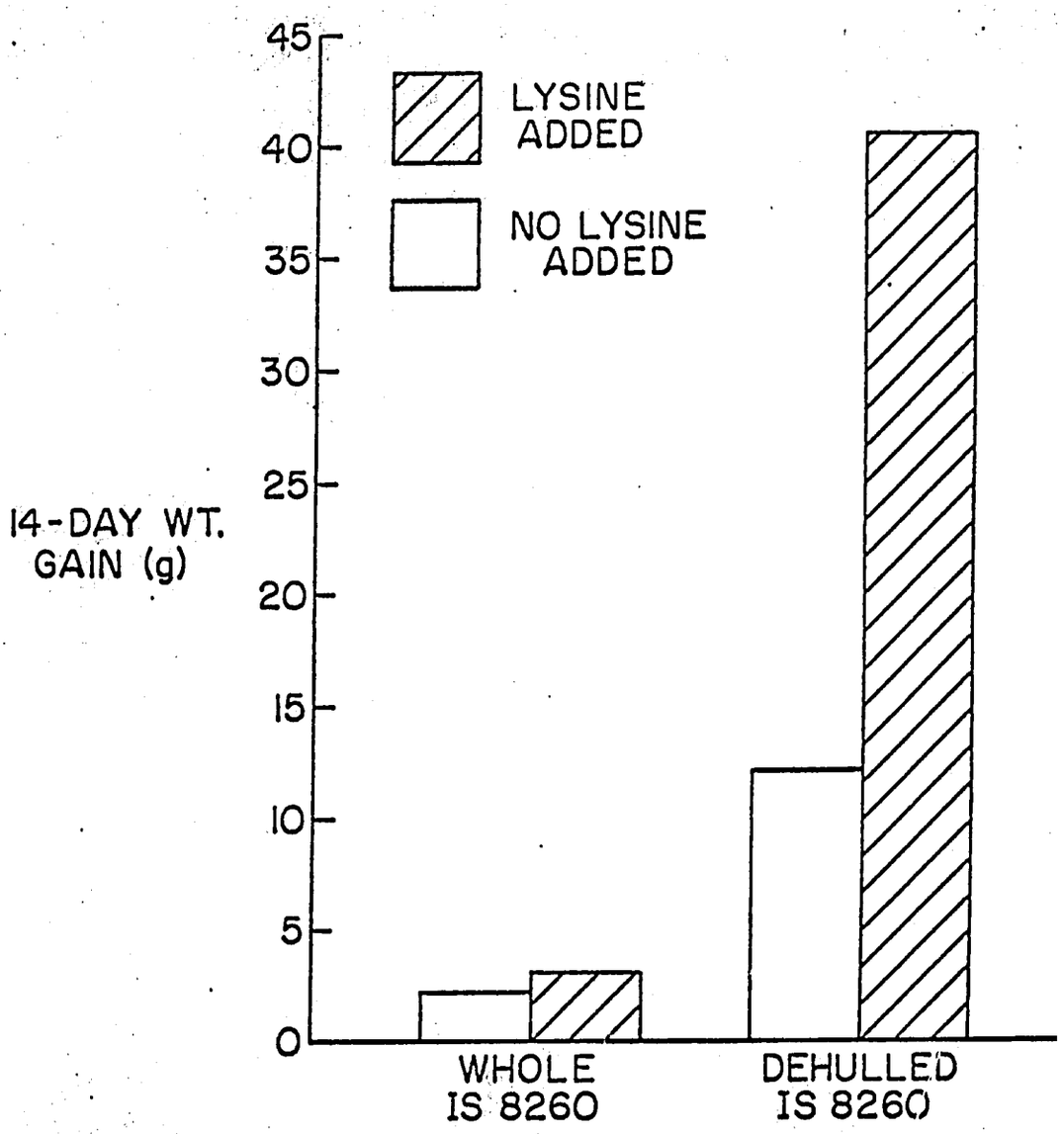

Figure 2. Comparison of 14-day weight gain of rats fed whole and dehulled sorghum diets with and without lysine added.

Table 8. Analysis of variance of NaOH dehulling and lysine supplementation of high tannin sorghum in a 14-day rat feeding experiment.

Source of Variation	df.	Mean Squares			
		Feed Consumption (g)	Weight Gain (g)	Feed Efficiency Ratios	Protein Efficiency Ratios
NaOH Dehulling	1	3940.2**	5640.6**	12601.4**	25.42**
Lysine Supplementation	1	1830.6**	2146.2**	420.5	6.18**
Rats	9	104.7	27.0	598.2*	0.07
Dehulling X Lysine	1	5313.0**	1918.2**	40.8	5.48**
Dehulling X Rats	9	229.2+	35.8+	596.7*	0.08+
Lysine X Rats	9	238.4+	25.1+	195.8+	0.02+
Error	9	139.8	17.6	202.2	0.05

* Significant at 5% level.
** Significant at 1% level.
\+ Pooled with error term for a better estimate of the true error.

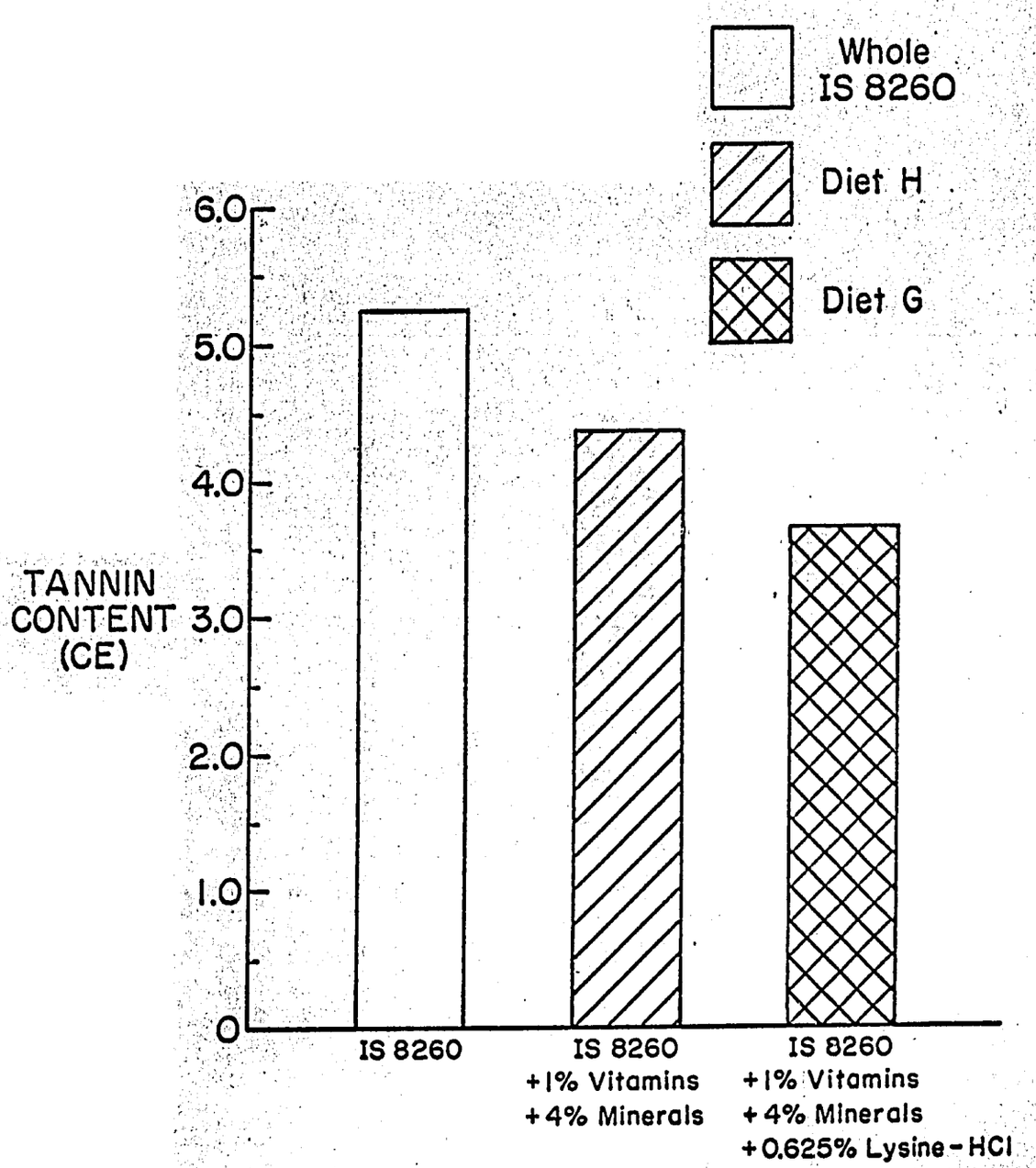

Figure 3. The effect of addition of vitamins, minerals and lysine-HCl on the CE level of sorghum diets.

Weanling Rat Response to Near Iso-nitrogenous Diets Differing in Tannin Content

Diets B, C, D, E, F and G (Table 1) represent a tannin gradient, ranging from 0.14 to 3.66 CE, at near iso-nitrogenous levels of protein (11.77 to 12.43 percent). Lysine was suboptimal in all diets varying from 69 to 75 percent of the rat requirement (Table 6). Data taken at 14 and 28 days show significant effects of CE levels on feed consumption, weight gain, feed efficiency ratios and protein efficiency ratios (Table 9). Mean weight gain on the high tannin (3.66 CE) diet (G) was only 9.0 grams in 28 days while the low tannin (0.14 CE) diet (B) supported a mean weight gain of 99.1 grams during the same period (Table 10), representing an 11-fold increase due to reduced tannin content. Figure 4 illustrates the effect of CE levels on 28-day weanling rat weight gain. The marked growth-depressing effects of low levels of tannin are shown and no threshold level is indicated. Some differences in feed consumption were noted especially for the two diets lowest in tannin (B and C). These differences are thought to be primarily due to differences in size of the rats resulting from different growth rates. This is supported by increased significant differences in mean feed consumption between groups after 28 days when compared to 14 days (Table 10). Feed efficiency ratios followed the expected trend although the differences were not significant except at the highest CE level. Protein efficiency ratios were significantly different at most CE levels. Protein efficiency ratios correlated much higher with other variables than feed efficiency ratios and were highly negatively correlated with CE levels (Table 11).

Table 9. Analysis of variance (mean squares) for CE levels in Experiment II.

Source of Variation	df.	Feed Consumption (g)	Weight Gain (g)	Feed Efficiency Ratios	Protein Efficiency Ratios
			14-Day Data		
CE Levels	5	2673.1**	1832.5**	1526.2**	7.1**
Error	54	199.2	33.2	124.2	0.1
			28-Day Data		
CE Levels		33630.6**	10757.9**	1126.2**	5.1**
Error		1658.6	249.3	231.9	0.1

** Significant at the 1% level.

In general, 14-day data correlated highly with 28-day data with FER having the lowest coefficient (r = 0.72**). This indicates that a researcher can successfully use 14-day feeding experiments requiring smaller quantities of samples and considerably less time and money to obtain information on the biological value of sorghum diets.

Table 10. Mean values[1] of variables measuring weanling rat growth response to sorghum diets at six levels of tannin (CE).

Diet	CE Level	14-Day Data				28-Day Data			
		Feed Consumption (g)	Weight Gain (g)	Feed Efficiency Ratios	Protein Efficiency Ratios	Feed Consumption (g)	Weight Gain (g)	Feed Efficiency Ratios	Protein Efficiency Ratios
B	0.14	120.4a[2]	40.7a	2.99a	2.70a	319.3a	99.1a	3.31a	2.45a
C	0.67	90.2b	20.4b	4.64a	1.80b	226.4b	50.4b	4.59a	1.76b
D	1.20	83.8b	13.0c	8.02a	1.20c	194.6bc	30.9c	6.71a	1.24c
E	1.49	79.0b	10.3c	9.26a	1.00cd	175.1c	22.6cd	9.00a	0.99cd
F	2.02	4b	7.1cd	14.99a	0.70d	177.2c	18.9cd	11.51a	0.88d
G	3.66	77.5b	3.1d	36.47b	0.37e	164.5c	9.0d	31.99b	0.45e

[1] Average of 10 rats.
[2] Any two means within a column not followed by the same letter differ at the 5% level of significance according to the Newman-Keuls test (Steel and Torrie, 1960).

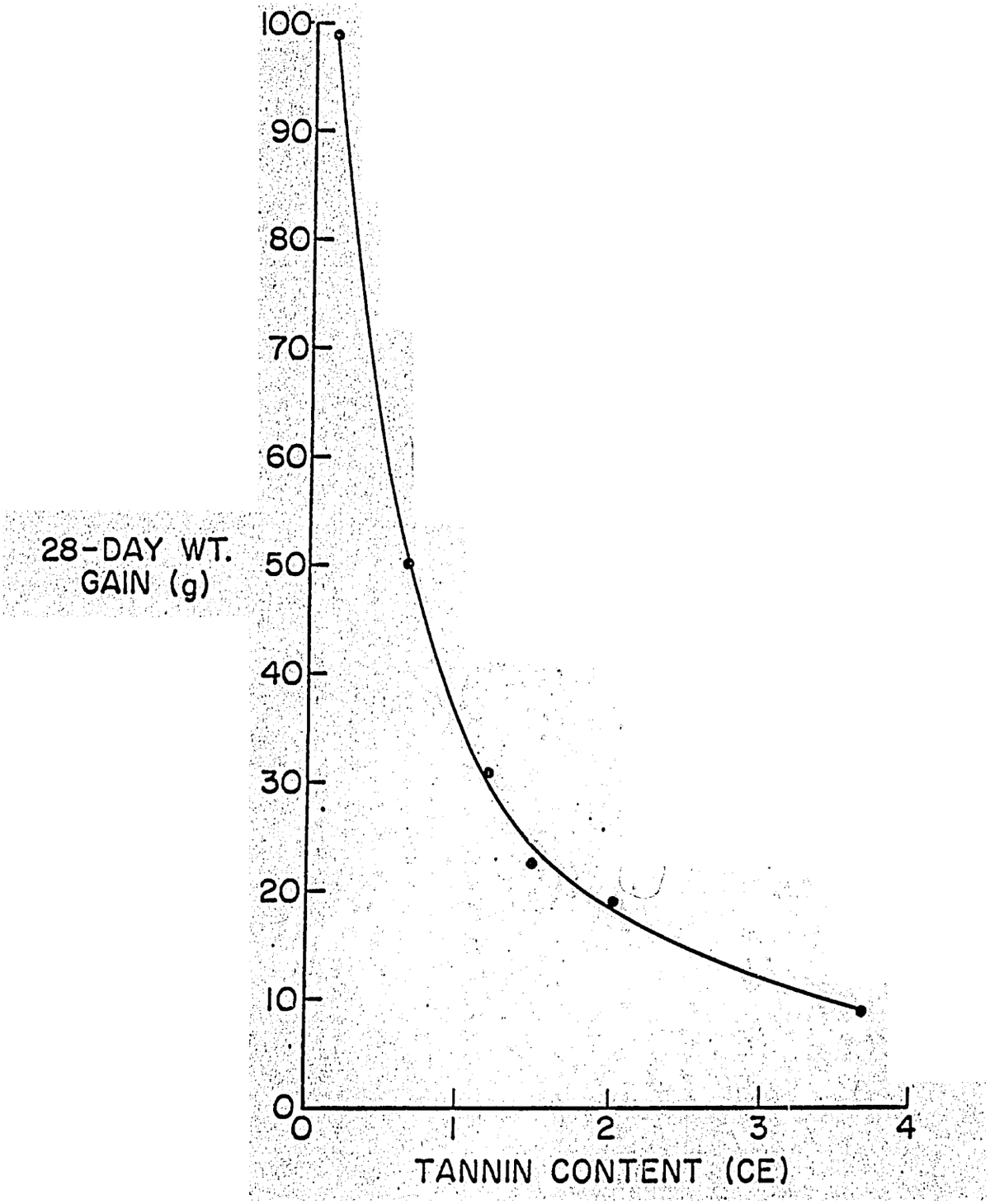

Figure 4. The effect of CE level on 28-day weanling rat weight gain.

Table 11. Correlation coefficients for CE and variables measuring weanling rat performance in Experiment II.

	CE	14-Day Data				28-Day Data			
		Feed Consumption	Weight Gain	FER	PER	Feed Consumption	Weight Gain	FER	PER
Catechin Equivalents	1.00	-0.53**	-0.76**	0.71**	-0.83**	-0.62**	-0.73**	0.54**	-0.84**
14-Day Data									
Feed Consumption		1.00	0.91**	-0.44**	0.81**	0.93**	0.85**	-0.31*	0.74**
Weight Gain			1.00	-0.56**	0.97**	0.93**	0.95**	-0.41**	0.91**
Feed Efficiency Ratios				1.00	-0.65**	-0.45**	-0.50**	0.72**	-0.65**
Protein Efficiency Ratios					1.00	0.85**	0.91**	-0.46**	0.95**
28-Day Data									
Feed Consumption						1.00	0.96**	-0.35**	0.85**
Weight Gain							1.00	-0.39**	0.93**
Feed Efficiency Ratios								1.00	-0.51**
Protein Efficiency Ratios									1.00

* Significantly different from zero at the 0.05 probability level.
** Significantly different from zero at the 0.01 probability level.

SUMMARY

Differences in catechin equivalent values for the VHCl and MVHCl tests were found. MVHCl values were generally higher and are thought to result from additional or different polyphenolic compounds being measured. The VHCl test was highly negatively correlated with weanling rat weight gain (r = -0.73**) and protein efficiency ratios (r = -0.84**), and is considered to be a useful tool for estimating sorghum quality. The presence or absence of the testa was shown to be an inaccurate indicator of nutritional quality since rats fed rations prepared from one group of bulked samples of grain with testa resulted in growth rates equivalent to rats fed diets prepared from grain lacking testa. The MVHCl test is considered less reliable for predicting sorghum nutritional quality since it is more highly correlated with the presence or absence of the testa than is the VHCl test.

Sodium hydroxide dehulling increased the biological value of high tannin sorghum for rats. Lysine supplementation was effective for improving rat performance on diets made from dehulled (low tannin) grain but no improvement was observed for whole-grain (high tannin) rations.

The use of a high tannin sorghum mixed in varying proportions with its dehulled counterpart enabled the formulation of a series of iso-nitrogenous diets differing only in tannin content. Weanling rat growth was inversely proportional to tannin content. The data indicated that

no threshold level of tannin is required for growth depression. Any amount of tannin in suboptimal protein diets would be expected to result in reduced nutritional quality. Data collected at 14 days and 28 days were highly correlated. It was concluded that there is no advantage in conducting the longer experiment at considerable additional cost for experiments of this type.

BIBLIOGRAPHY

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Armstrong, W. D., W. R. Featherston and J. C. Rogler. 1973. Influence of methionine and other dietary additions on the performance of chicks fed bird resistant sorghum grain diets. Poul. Sci. (In Press).

Axtell, J. D., D. L. Oswalt, E. T. Mertz, R. C. Pickett, R. Jambunathan and G. Shrinivasan. 1973. Components of nutritional quality in grain sorghum. Proc. of the Second Int'l Symp. on Protein Quality in Maize, El Baton, Mexico, Dec. 1972 (In Press).

Bate-Smith, E. C. and V. Rasper. 1969. Tannins of grain sorghum: Luteoforol (leucoluteolinidin), 3', 4, 4', 5, 7-pentahydroxyflavin. J. Food Sci. 34:203-209.

Blessin, C. W. 1971. Processing sorghum grain to improve nutritional quality and industrial use. Seventh Biennial Grain Sorghum Research and Utilization Conference. p. 70.

Blessin, C. W., C. H. Van Etten and R. J. Dimler. 1963. An examination of anthocyanogens in grain sorghums. Cereal Chem. 40:241-250.

Burns, R. E. 1963. Methods of tannin analysis for forage crop evaluation. Tech. Bull. N. S. 32. Ga. Agr. Exp. Sta., Athens, Ga.

Burns, R. E. 1971. Method of estimation of tannin in grain sorghum. Agron. J. 63:511-512.

Chang, S. I. and H. L. Fuller. 1964. Effect of tannin content of grain sorghums on their feeding value for growing chicks. Poul. Sci. 43:32-36.

Connor, J. K., I. S. Hurwood, H. W. Burton and D. E. Fuelling. 1969. Some nutritional aspects of feeding sorghums of high tannin content to growing chickens. Austral. J. Exp. Agric. Animal Husb. 9:497-501.

Fuller, H. L., D. K. Potter and A. R. Brown, 1966. The feeding value of grain sorghums in relationship to their tannin content. Tech. Bull. N. S. 176. Ga. Agr. Exp. Sta., Athens, Ga.

Glick, Z. and M. A. Joslyn. 1970. Effect of tannic acid and related compounds on the absorbtion and utilization of protein in rats. J. Nutrition 100:516-520.

Goldstein, J. L. and T. Swain. 1965. The inhibition of enzymes by tannins. Phytochem. 4:185-192.

Handler, P. and R. D. Baker. 1944. Toxicity of orally administered tannic acid. Science 99:393-394.

Harrington, G. T. and W. Crocker. 1923. Structure, physical characteristics and composition of the pericarp and integument of Johnson grass seed in relation to its physiology. J. Agr. Res. 23:193-222.

Harris, H. B. 1969. Bird resistance in grain sorghum. Proc. 24th Ann. Corn and Sorghum Res. Conf. Chicago, Ill. pp. 113-122.

Harris, H. B. and R. E. Burns. 1970. Influence of tannin content on preharvest seed germination in sorghum. Agr. J. 62:835-836.

Harris, H. B., B. J. Johnson and S. V. Stacy. 1964. Georgia 615 hybrid grain sorghum. Ga. Exp. Sta. Lft. No. N. S. 43:1-7.

Jambunathan, R. and E. T. Mertz. 1973. Relationship between tannin levels, rat growth, and distribution of proteins in sorghum. J. Agr. Food Chem. 21:692-696.

Joslyn, M. A. and Z. Glick. 1969. Comparative effects of gallotannic acid and related phenolics on the growth of rats. J. Nutr. 98:119-126.

Lease, E. J. and J. H. Mitchell. 1940. A study on the tannins of Lespedeza sericea. So. Car. Agr. Exp. Sta. Ann. Rep. 53:710.

Maxson, E. D. and L. W. Rooney. 1972. Evaluation of methods for tannin analysis in sorghum grain. Cereal Chem. 49:719-729.

Maxson, E. D., L. E. Clark, L. W. Rooney and J. W. Johnson. 1972. Factors affecting the tannin content of sorghum grain as determined by two methods of tannin analysis. Crop Sci. 12:233-235.

McGinty, D. D. 1968a. Factors affecting the digestibility of varieties of sorghum grain by cattle. Ph. D. Thesis. Texas A & M University, College Station, Texas.

McGinty, D. D. 1968b. Variation in digestibility of sorghum varieties Proc. 6th Biennial Int. Grain Sorghum Res. and Utilization Conf. (Amarillo, Tex., March 5-7, 1969):20-23.

McMillian, W. W., B. R. Wiseman, R. E. Burns, H. B. Harris and G. L. Greene. 1972. Bird resistance in diverse germplasm of sorghum. Agr. J. 64:821-822.

Mertz, E. T. 1969. Amino acid and protein requirements of fish. Fish in Research, Acad. Press., Inc. New York.

Miller, B. S. and E. Kneen. 1947. The amylase inhibitor of Leoti sorghum. Arch. Biochem. 15:251-264.

Morton, J. R. 1970. Tentative correlations of plant usage and esophageal cancer zones. Econ. Bot. 24:217-226.

Niehaus, M. H. and W. H. Schmidt. 1970. Evaluation of bird resistant grain sorghum in Ohio. Agron. J. 62:677-678.

Oswalt, D. L. 1973. Nutritional quality of Sorghum bicolor (L.) Moench as estimated by polyphenols, crude protein, amino acid composition and rat performance. Ph. D. Thesis. Purdue University, West Lafayette, Indiana.

Quinby, J. R. and J. H. Martin. 1954. Sorghum improvement. Advances in Agron. Acad. Press, New York 6:305-359.

Rayudu, G. V. N., R. Kadirvel, P. Vohra and F. H. Kratzer. 1970. Toxicity of tannic acid and its metabolites for chickens. Poul. Sci. 49:957-960.

Ringrose, R. C. and C. L. Morgan. 1940. The nutritive value of Lespedeza for poultry feeding. So. Car. Exp. Sta. Ann. Rep. 53:91-92.

Rostango, H. S., W. R. Featherston and J. C. Rogler. 1973a. Studies on the nutritional value of sorghum grains with varying tannin contents for chicks. 1. Growth studies. Poul. Sci. 52:765-772.

Rostango, H. S., J. C. Rogler and W. R. Featherston. 1973b. Studies on the nutritional value of sorghum grain with varying tannin contents for chicks. 2. Amino acid digestibility studies. Poul. Sci. 52:772-778.

Roux, D. G. 1957. Some recent advances in the identification of leucoanthocyanins and the chemistry of condensed tannins. Nature 180:973-975.

Roux, D. G. and S. R. Evelyn. 1958. Condensed tannins. I. A study of complex leucoanthocyanins present in condensed tannins. Biochem. J. 69:530-538.

Schaffert, R. E. 1972. Protein quantity, quality, and availability in Sorghum bicolor (L.) Moench grain. Ph. D. Thesis. Purdue University, West Lafayette, Indiana.

Smart, W. W. G. Jr., T. A. Bell, N. W. Stanley and W. A. Cope. 1961. Inhibition of rumen cellulase by an extract from Sericea forage. J. Dairy Sci. 44:1945-1946.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures in statistics. McGraw-Hill Book Co., Inc., New York.

Strumeyer, D. H. and M. J. Malin. 1969. Identification of the amylase inhibitor from seeds of Leoti Sorghum. Biochem et. Biophys. Acta 184:643-645.

Tamir, M. and E. Alumont. 1969. Inhibition of digestive enzymes by condensed tannins from green and ripe carobs. J. Sci. Food Agric. 20:199-202.

Tamir, M. and E. Alumont. 1970. Carob tannins - growth depression and levels of insoluable nitrogen in the digestive tract of rats. J. Nutrition 100:573-580.

Tipton, K. W., E. H. Floyd, J. G. Marshall and J. B. McDevitt. 1970. Resistance of certain grain sorghum hybrids to bird damage in Louisiana. Agron. J. 62:211-213.

Vohra, P., F. H. Kratzer and M. A. Joslyn. 1966. The growth depressing and toxic effects of tannins on chicks. Poul. Sci. 45:135-142.

Voigt, R. L. 1966. Bird tolerant sorghums boost take-home yields. Prog. Agr. Ariz. 18:30-32.

Wall, J. S. and W. M. Ross. 1970. Sorghum production and utilization. Avi. Pub. Co., Westport, Connecticut.

Wanjari, M. R. and J. O. York. 1972. Inheritance of brown pericarp and subcoat in sorghum. Crop Sci. 12:819-821.

York, J. O. and R. L. Thurman. 1962. Aks 614 hybrid grain sorghum. Ark. Farm Res. 11:5.

VITA

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